

## NUCLEIC ACID SEQUENCES FROM *DROSOPHILA MELANOGASTER* THAT ENCODE PROTEINS ESSENTIAL FOR VIABILITY AND USES THEREOF

This application claims the benefit of United States Provisional Patent Application Serial No. 60/422,377 filed October 30, 2002, which is incorporated by reference in its entirety.

The Sequence Listing associated with the instant disclosure has been submitted as a 2.62 megabyte file on CD-R (in duplicate) instead of on paper. Each CD-R is marked in indelible ink to identify the Applicants, Title, File Name (70131WOPCT.ST25.txt), Creation Date (August 7, 2003), Computer System (IBM-PC/MS-DOS/MS-Windows), and Docket No. (70131WOPCT). The Sequence Listing submitted on CD-R is hereby incorporated by reference into the instant disclosure.

### FIELD OF INVENTION

The present invention pertains to nucleic acid sequences isolated from *Drosophila melanogaster* that encode proteins essential for viability. The invention particularly relates to methods of using these proteins as insecticide targets, based on this essentiality.

### BACKGROUND OF THE INVENTION

Insects contribute or cause many human and animal diseases, and are responsible for substantial agricultural and property damage. The societal costs associated with insect pests in dollars, time and suffering are monumental. The total worldwide market size for insecticide crop protection is over \$5 billion. To combat these problems, insecticidal compounds have been developed and employed.

The idea to use chemicals for insect control is not new. The scientific use of pesticides started with the introduction of arsenical insecticides and organic compounds such as tar, petroleum oils, and dinitrophenol emulsions at the end of the last century. But, the systematic search for synthetic organic insecticides was only launched after the discovery of the insecticidal properties of DDT in 1939. After World War II, chemical research concentrated mainly on chlorinated hydrocarbons and cyclodienes, which all require high rates of application and have a rather broad spectrum of activity. Most of them are persistent in the environment and may pose a significant risk for accumulation in the food chain. Today the use of these chemicals is very much restricted.

From this point, the major emphasis in research has been given to organophosphates and carbamates, which are readily degradable in the environment with little tendency for bioaccumulation. The toxicity of these compounds varies within a broad range from medium to highly toxic. Organophosphates and carbamates are still widely use, although the more toxic ones are banned in certain countries. The formamidines have as their major advantage a different mode of action and their selectivity, which made them suitable for use in IPM (insect pest management) programs. They are easily degradable with no accumulation potential, but for toxicological reasons some have had to be withdrawn from the market.

For the past decade, insecticide research has concentrated on leadfinding for new chemical structures interfering with new target mechanisms. The chances for success are rather remote, because the hurdles for the registration of a new insecticide are set very high. Toxicological aspects, insecticide resistance, environmental behavior, and IPM fitness are some of the critical factors that have to be considered together with economical factors.

Novel insecticides can now be discovered using high-throughput screens that implement recombinant DNA technology. Proteins found to be essential to insect viability can be recombinantly produced through standard molecular biological techniques and utilized as insecticide targets in screens for novel inhibitors of the enzymes' activity. The novel inhibitors discovered through such screens may then be used as insecticides to control undesirable insect infestation.

However, as the world population continues to grow, there will be increasing food shortages. Therefore, there exists continuing need to find new, effective and economic insecticides.

#### SUMMARY OF THE INVENTION

In view of these needs, it is one object of the invention to provide essential genes in insects such as *Drosophila melanogaster*. It is another object to provide the essential proteins encoded by these essential genes for assay development to identify inhibitory compounds with insecticidal activity. It is still another object of the present invention to provide an effective and beneficial method for identifying new or improved insecticides using the essential proteins of the invention.

In furtherance of these and other objects, the present invention provides DNA molecules comprising nucleotide sequences isolated from *Drosophila melanogaster* that encode proteins

essential for viability. The inventors are the first to demonstrate that the nucleotide sequences of the invention are essential for viability. This knowledge is exploited to provide novel insecticide modes of action. One advantage of the present invention is that the proteins encoded by the essential nucleotide sequences provide the bases for assays designed to easily and rapidly identify novel insecticides.

Disruption of the nucleotide sequences or messenger RNA of the invention demonstrates that the activity of each corresponding encoded protein is essential for *Drosophila* viability. Genetic results show that when each nucleotide sequence of the invention is mutated in *Drosophila* or disrupted at the transcription level, the resulting phenotype is lethal.. This demonstrates a critical role for the protein encoded by the mutated nucleotide sequence. This further implies that chemicals that inhibit the expression of the protein when in contact with insects are likely to have detrimental effects on insects and are potentially good insecticide candidates. The present invention therefore provides methods of using the disclosed nucleotide sequences or proteins encoded thereby to identify inhibitors thereof. The inhibitors can then be used as insecticides to kill undesirable insect populations where crops are grown, particularly agronomically important crops such as maize, and other cereal crops such as wheat, oats, rye, sorghum, rice, barley, millet, turf and forage grasses and the like, as well as cotton, sugar cane, sugar beet, oilseed rape, soybeans, vegetable crops and fruits.

The present invention accordingly provides cDNA sequences derived from *Drosophila melanogaster*. In one embodiment, the present invention provides an isolated DNA molecule comprising a nucleotide sequence selected from the group consisting of the even numbered SEQ ID NOs:14-380. In another embodiment, the present invention provides an isolated DNA molecule comprising a nucleotide sequence that encodes a protein selected from the group consisting of the odd numbered SEQ ID NOs:15-381.

The present invention also provides a chimeric construct comprising a promoter operatively linked to a DNA molecule according to the present invention, wherein the promoter is preferably functional in a eukaryote, wherein the promoter is preferably heterologous to the DNA molecule. The present invention further provides a recombinant vector comprising a chimeric construct according to the present invention, wherein said vector is capable of being stably transformed into a host cell. The present invention still further provides a host cell comprising a DNA

molecule according to the present invention, wherein said DNA molecule is preferably expressible in the cell. The host cell is preferably selected from the group consisting of an insect cell, a yeast cell, and a prokaryotic cell.

The present invention also provides proteins essential for *Drosophila melanogaster* viability. In one embodiment, the present invention provides an isolated protein comprising an amino acid sequence selected from the group consisting of the odd numbered SEQ ID NOs:15-361. In accordance with another embodiment, the present invention also relates to the recombinant production of proteins of the invention and methods of using the proteins of the invention in assays for identifying compounds that interact with the protein.

In another preferred embodiment, the present invention describes a method for identifying chemicals having the ability to inhibit the activity of the disclosed proteins. In a preferred embodiment, the present invention provides a method for selecting compounds that interact with a protein of the invention, comprising: (a) expressing a DNA molecule according to the present invention to generate the corresponding protein of the invention, (b) testing a compound suspected of having the ability to interact with the protein expressed in step (a), and (c) selecting compounds that interact with the protein in step (b).

Other objects and advantages of the present invention will become apparent to those skilled in the art and from a study of the following description of the invention and non-limiting examples. The entire contents of all publications mentioned herein are hereby incorporated by reference.

#### BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

SEQ ID NOs:1-13 are PCR primers.

Even numbered SEQ ID NOs:14-380 are nucleotide sequences described in the table below.

Odd numbered SEQ ID NOs:15-381 are protein sequences encoded by the immediately preceding nucleotide sequence, e.g., SEQ ID NO:15 is the protein encoded by the nucleotide sequence of SEQ ID NO:14, SEQ ID NO:17 is the protein encoded by the nucleotide sequence of SEQ ID NO:16, etc.

Table 1      *Drosophila* Sequences

seq ID	Inventor's reference	function	Domains	Best blast hit	score
14-15	CT28483	CG10260 EG:BACR7C10.2 protein kinase, 1-phosphatidylinositol 4-kinase	PI3Ka, PI3_4_KINASE_1, PI3_4_KINASE_2, PI3_4_KINASE_3, PI3_PI4_kinase	(D83538) 230kDa phosphatidylinositol 4-kinase [Rattus norvegicus]	1600
16-17	CT28925	CG10365 unknown		hypothetical protein MGC4504 [Homo sapiens]	185
18-19	CT29122	CG10370 Tbp-1 Tat-binding protein-1, Proteasome 26S regulatory subunit 6A, multicatalytic endopeptidase,	AAA, ATP_GTP_A, MITOCH_CARRIER	Q63569[PRSA_RAT 26S PROTEASE REGULATORY SUBUNIT 6A (TAT-BINDING PROTEIN 1) (TBP-1)]	720
20-21	CT29492	CG10545 Gb13F G protein b-subunit 13F, G-protein coupled receptor, protein signaling pathway	GPROTEINB, GPROTEINBRPT, WD40, WD40_REGION, WD_REPEATS	GBB1 CAEEL GUANINE NUCLEOTIDE-BINDING PROTEIN BETA SUBUNIT 1	619
22-23	CT30008	CG10701 Moe Dmoesin, motor involved in cytoskeleton organization and biogenesis	BAND41, BAND_41_1, BAND_41_2, BAND_41_3, Band_41, ERM, ERMFAMILY	Homo sapiens 'moesin' gi:4505257	
24-25	CT30208	CG10776 wit Serine/threonine kinase-D; wishful thinking, a type II transforming growth factor beta receptor involved in protein phosphorylation	PROTEIN_KINASE_ATP, PROTEIN_KINASE_DOM, TGFB_RECEPTOR, pkinase	NP_031587.1  (NM_007561) bone morphogenic protein receptor, type II	362
26-27	CT30807	CG10997 chloride channel?		NP_001280.2  (NM_001289) chloride intracellular channel 2 [Homo sapiens]	119
28-29	CT30887	CG11033 unknown		NP_036440.1  (NM_012308) F-box and leucine-rich repeat protein 11	431
30-31	CT31117	CG11130 Rtc1 RNA 3' terminal phosphate cyclase, Rtc1		Q9Y2P8 RCL1_HUMAN RNA 3'-TERMINAL PHOSPHATE CYCLASE-LIKE PROTEIN (HSPC338)	326
32-33	CT1249	CG1114 Weak similarity with apoptosis protein RP-8,		NP_071334.1  (NM_022051) egl nine homolog 1 (C. elegans)	249
34-35	CT1483	CG1119 Gnfl Germ line transcription factor 1, DNA binding/DNA replication factor	ATP_GTP_A, BRCT, BRCT_DOMAIN, NLS_BP, RFC	A49651 replication factor C large subunit - human	661
36-37	CT7860	CG11190 unknown		BAB60854.1  (AB057724) phosphatidyl inositol glycan class T [Homo sapiens]	387

38-39	CT1834	CG1135 unknown	FHA, FHA_DOMAIN	NP_006328.1  (NM_006337) microspherule protein 1; cell cycle-regulated factor	383
40-41	CT31875	CG11418 EG:8D8.8 involved in cell cycle		NP_060579.1  (NM_018109) hypothetical protein FLJ10486 [Homo sapiens]	252
42-43	CT36241	CG11452 unknown		none	
44-45	CT1993	CG1149 MstProx MstProx, transmembrane receptor involved in defense response	LRRNT	Homo sapiens 'toll-like receptor1' gi:4507527	
46-47	CT34608	CG11511 similarity to broad-complex Z2-isoform	ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	AAC78286.1  (AF032674) broad-complex Z2-isoform [Manduca sexta]	128
48-49	CT5404	CG11595 unknown		none	
50-51	CT17728	CG11779 receptor - mitochondrial transporter???		XP_049282.1  (XM_049282) translocase of inner mitochondrial membrane 44 homolog	436
52-53	CT1465	CG12007 geranylgeranyltransferase, alpha subunit		NP_004572.1  (NM_004581) Rab geranylgeranyltransferase, alpha subunit [Homo sapiens]	278
54-55	CT5438	CG12079 NADH dehydrogenase (ubiquinone)	complex1_30Kd	AAD40386.1  (AF100743) NADH-Ubiquinone reductase [Homo sapiens]	323
56-57	CT43008	CG12085 pUbsf DPUF68 Puf60 polyU binding splicing factor, poly(U) binding involved in mRNA splicing	RBD, RNP_1, rrm	NP_525123.1  (NM_080384) poly-U-binding splicing factor	1037
58-59	CT5902	CG12093 unknown	CRYSTALLIN_BETAGAMMA	NP_499515.1  (NM_067114) Y41C4A.8.p [Caenorhabditis elegans]	137
60-61	CT6734	CG12113 unknown	ATP_GTP_A	AAH08013 (BC008013) Similar to CG12113 gene product [Homo sapiens]	498
62-63	CT7760	CG12135 c12.1 unknown		AF110775_1 (AF110775) adrenal gland protein AD-002 [Homo sapiens]	252
64-65	CT9355	CG12181 Sgs4 sgs-4 salivary gland secretion protein 4, pupal glue protein		Mus musculus Sap62 MGI:104912	
66-67	CT12665	CG12225 Spt6 spt6, promoter-associated pausing and transcriptional elongation	S1	Caenorhabditis elegans T04A8.14 WP:CE13120	
68-69	CT13424	CG12238 'probable transcription factor		NP_060758.1  (NM_018288) hypothetical protein FLJ10975 [Homo sapiens]	222

70-71	CT14932	CG12251 AQP AQP aquaporin, water channel		XP_059490.1  (XM_059490) hypothetical protein XP_059490 [Homo sapiens]	62.4
72-73	CT23511	CG12348 Sh open rectifying potassium channel, shaker			
74-75	CT32757	CG12482 unknown		NP_076113.1  (NM_023624) lecithin-retinol acyltransferase [Mus musculus]	40.8
76-77	CT33237	CG12497 EG:BACR25B3.2 low-density lipoprotein receptor-like	LDLRA_1, LDLRA_2, LDLRECEPTOR, NLS_BP, PRO_RICH, ldl_recept_a	CAC86027.1  (AJ313389) tsetse EP protein [Glossina morsitans morsitans]	90.9
78-79	CT33996	CG12537 unknown		AAK31375.1 AC084329_1 (AC084329) ppg3 [Leishmania major]	116
80-81	CT34671	CG12600 unknown	WW_rsp5_WWP	AF213258_1 (AF213258) membrane-associated guanylate kinase-related MAGI-3 [Mus musculus]	56.2
82-83	CT2591	CG1265 unknown		XP_059471.1  (XM_059471) similar to MANNOSE-P-DOLICHOL UTILIZATION DEFECT 1	67.8
84-85	CT35764	CG12701 unknown	NLS_BP, PRO_RICH, ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	NM_078717  kismet [Drosophila melanogaster]	117
86-87	CT28931	CG12750 nucampholin, transcription factor?	RNA binding	(AB046824) KIAA1604 protein [Homo sapiens]	833
88-89	CT32253	CG13034 unknown		(AC084329) ppg3 [Leishmania major]	94.4
90-91	CT32701	CG13372 EG:171D11.6 unknown		none	
92-93	CT40992	CG13372 EG:171D11.6 unknown		none	
94-95	CT32721	CG13380 unknown		NP_499428.1  (NM_067027) W09D6.5.p [Caenorhabditis elegans]	43.5
96-97	CT33014	CG13620 unknown	CYTOCHROME_C, NLS_BP, ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	Caenorhabditis elegans 'similar to Zinc finger, C2H2 type	
98-99	CT33019	CG13625 histone protein?	NLS_BP	NP_498982.1  (NM_066581) R08D7.1.p [Caenorhabditis elegans]	265
100-101	CT33241	CG13760 EG:BACR25B3.6 unknown	<u>Cysteine proteinases</u>	(AK054681) unnamed protein product [Homo sapiens]	144
102-103	CT33317	CG13818 unknown	ATP_GTP_A	T26047 hypothetical protein W01C8.5 - Caenorhabditis elegans	39.3

104-105	CT3228	CG1405 cg1405 ATP dependent helicase	HELICASE, helicase_C	XP_008088.1  (XM_008088) pre-mRNA splicing factor Prp16 [Homo sapiens]	825
106-107	CT33819	CG14206 structural protein of ribosome		AF400207_1 (AF400207) ribosomal protein S10 [Spodoptera frugiperda]	225
108-109	CT3352	CG1422 p115 vesicular transporter, membrane docking		P41541 VDP_BOVIN General vesicular transport factor p115	725
110-111	CT33841	CG14226 CT33841 protein tyrosine phosphatase	fn3	NP_075214.1  (NM_022925) protein tyrosine phosphatase, receptor type, Q [Rattus]	93.6
112-113	CT34063	CG14411 protein phosphatase	CRYSTALLIN_BETAGAMMA	AAK26171.1  (AY028703) phosphatidylinositol-3 phosphate 3-phosphatase adaptor	211
114-115	CT3509	CG1448 inx3 innexin 3		Q9XYN1 INX2_SCHAM Innexin Inx2 (Innexin-2) (G-Inx2)	332
116-117	CT34434	CG14656 unknown		NP_542443.1  (NM_080712) tty-P1 [Drosophila melanogaster]	122
118-119	CT34588	CG14778 integral peroxisomal membrane		(AE003604) CG2022 gene product [Drosophila melanogaster]	179
120-121	CT43287	CG14779 EG:80H7.2 tubulin-beta mRNA autoregulation signal protein	Tubulin-beta mRNA autoregulation signal domain	none	
122-123	CT34589	CG14779 EG:80H7.2 tubulin-beta mRNA autoregulation signal protein	Tubulin-beta mRNA autoregulation signal domain	none	
124-125	CT34599	CG14789 EG:BACN32G11.6 Aminoacyl-transfer RNA synthetases class-I signature protein	AA_TRNA_LIGASE_I	AF455270_1 (AF455270) C21ORF80 [Mus musculus]	261
126-127	CT34602	CG14792 sta Laminin-receptor Stubarista, protein biosynthesis Rp40	RIBOSOMALS2, RIBOSOMAL_S2_1, RIBOSOMAL_S2_2, Ribosomal S2	(AB032438) stubarista [Drosophila erecta]	410
128-129	CT34626	CG14813 delta;COP coatomer complex COPI delta-COP subunit delta	ATP_GTP_A: ATP/GTP-binding site motif A (P-loop) protein	NP_001646.2  (NM_001655) archain; coatomer protein delta-COP [Homo sapiens]	585
130-131	CT34665	CG14849 unknown		none	
132-133	CT3729	CG1489 Pros45 sug1, multicatalytic endopeptidase regulator, multicatalytic endopeptidase, , proteasome ATPase, proteolysis and	AAA, ATP_GTP_A	P54814 PRS8_MANSE 26S PROTEASE REGULATORY SUBUNIT 8 (18-56 PROTEIN)	727



		peptolysis			
134- 135	CT34842	CG14991 unknown	BAND_41_3, PH_DOMAIN	XP_051693.1  (XM_051693) mitogen inducible 2 [Homo sapiens]	635
136- 137	CT34979	CG15104 topoisomerase I-binding RS protein'		NP_055023.1  (NM_014208) dentin sialophosphoprotein; dentin phosphophoryn;	102
138- 139	CT3955	CG1530 unknown	PRO_RICH	XP_092523.1  (XM_092523) hypothetical protein XP_092523 [Homo sapiens]	230
140- 141	CT35308	CG15321 unknown		none	
142- 143	CT35676	CG15560 putative cell membrane-associated mucin		NP_499205.1  (NM_066804) Transmembrane and sushi domain [Caenorhabditis elegans]	170
144- 145	CT30180	CG15811 Rop rop, 'Ras opposite	Sec1	NP_037170.1  (NM_013038) syntaxin binding protein 1 [Rattus norvegicus]	756
146- 147	CT34113	CG15896 unknown		NP_055487.1  (NM_014672) KIAA0391 gene product [Homo sapiens]	182
148- 149	CT34115	CG15898 unknown		NP_078828.1  (NM_024552) hypothetical protein FLJ12089 [Homo sapiens]	47.8
150- 151	CT4708	CG1683 Ant2 Ant2, ADP/ATP translocase. Adenine nucleotide translocase 2, ATP/ADP antiporter	ADPTRNSLCASE, MITOCARRIER, MITOCH_CARRIER, mito_carr	(AF218587) ADP/ATP translocase [Lucilia cuprina]	485
152- 153	CT37506	CG16903 EG-67A9.2 non-specific RNA polymerase II transcription factor		NP_446114.1  (NM_053662) cyclin L [Rattus norvegicus]	411
154- 155	CT35131	CG16916 Rpt3 p48A, 26S proteasome regulatory complex subunit p48A	AAA, CLPPROTEASEA	PRS6_MANSE 26S PROTEASE REGULATORY SUBUNIT 6B (ATPASE MS73)	681
156- 157	CT4802	CG1696 unknown		NP_056158.1  (NM_015343) hypothetical protein [Homo sapiens]	341
158- 159	CT43084	CG1697 rho-4 rho-4 Rho-related [10C6] rhomboid-4		<u>Rattus norvegicus 'rhomboid-related protein'</u> <u>EMBL:Y17258</u>	
160- 161	CT4810	CG1698 unknown		none	
162- 163	CT4826	CG1703 ATP-binding cassette (ABC) transporter	ABC_TRANSPORTER, ABC_tran, ATP_GTP_A, ATP_GTP_A2, DA_BOX, NLS_BP	(AF293383) ABC50 [Rattus norvegicus]	802

164-165	CT35402	CG17252 BCL7-like BCL7-like		(NM_001707) B-cell CLL/lymphoma 7B [Homo sapiens]	94.4
166-167	CT21145	CG17309 CSK CSK, involved in protein phosphorylation	PROTEIN_KINASE_ATP, PROTEIN_KINASE_DOM, PROTEIN_KINASE_TYR, SH2, SH2DOMAIN, TYRKINASE, pkinase	AAH18394 (BC018394) c-src tyrosine kinase [Mus musculus]	462
168-169	CT5050	CG1740 Ntf-2 NTF-2, protein carrier involved in protein-nucleus import	NTF2_DOMAIN	(NM_059921) nuclear transport factor 2 like [Caenorhabditis]	127
170-171	CT5086	CG1746 anon- EST:Posey224 hydrogen- transporting ATP synthase/enzyme, hydrogen-transporting two-sector ATPase	ATP-synt_C, ATPASEC, ATPASE_C	Q9U505[ATPC_MANSE ATP synthase subunit C, mitochondrial precursor (Lipid- binding	177
172-173	CT34491	CG17734 unknown		NP_062788.1 (NM_019814) hypoxia induced gene 1 [Mus musculus]	82.4
174-175	CT39345	CG17766 EG:86E4.3 heterotrimeric G-protein GTPase	WD40, WD40_REGION	AF188123_1 (AF188123) TGF- beta resistance-associated protein TRAG [Mus musculus]	1160
176-177	CT39414	CG17791 sqd heterogeneous-nuclear- ribonucleoprotein-87Fb RNA-binding protein 3, Squid	RBD, rrm; Eukaryotic putative RNA-binding region RNP-1 signature, RRM-motif protein, RRM-motif protein	Homo sapiens 'heterogeneous nuclear ribonucleoprotein D' EMBL:AF026126	
178-179	CT39758	CG17871 Or71a tracheal gasfilling mutant1b, Or71a, odorant receptor		none	
180-181	CT40282	CG18009 Trf2 TATA box binding protein-related factor 2		(AB024489) TBP-like protein [Gallus gallus]	210
182-183	CT5456	CG1826 product involved in developmental processes	BTB, NLS_BP, PROTEIN_SPLICING	(AB067467) KIAA1880 protein [Homo sapiens]	595
184-185	CT41472	CG18282 Ubiquitin-like		I45964 polyubiquitin - bovine (fragment)	431
186-187	CT42468	CG18578 Ugt86Da UDP- glucuronosyltransferase		none	
188-189	CT13908	CG18734 Fur2 furin		T43251 furin (EC 3.4.21.75) - fall armyworm	1753
190-191	CT5890	CG1908 unknown	NLS_BP	none	
192-193	CT5932	CG1915 sls sallimus, myosin light chain kinase	AA_TRNA_LIGASE_II_1, ATP_GTP_A, NLS_BP, SH3, fn3, ig	Gallus gallus 'connectin/titin' EMBL:D83390	

194-195	CT6007	CG1937 involved in cell growth and maintenance		(AF317634) HRD1 [Homo sapiens]	545
196-197	CT5951	CG1938 Dlic2 Dlic2, motor which is a component of the microtubule associated protein	ATP_GTP_A	(AF317841) cytoplasmic dynein light-intermediate chain 1 [Xenopus]	399
198-199	CT6352	CG1994 similar to Achlya ambisexualis antheridiol steroid receptor	ATP_GTP_A	(AB051496) KIAA1709 protein [Homo sapiens]	1013
200-201	CT6373	CG2003 high affinity inorganic phosphate:sodium symporter	transporter	Homo sapiens 'Na/PO4 cotransporter' gi:4885441	
202-203	CT4336	CG2151 Trxr-1 NOT glutathione reductase (NADPH) (EC:1.6.4.2) involved in thioredoxin reduction	FADPNR, HGRDTASE, NAD_BINDING, PNDRDTASE1, PYRIDINE_REDOX_1, pyr redox	(U88187) glutathione reductase family member [Musca domestica]	753
203-205	CT6738	CG2165 BEST:CK01140 calcium-transporting ATPase-like		(NM_053311) ATPase, Ca++ transporting, plasma membrane 1 [Rattus]	1262
206-207	CT5965	CG2184 Mlc2 muscle-specific myosin regulatory light chain Mlc2, involved in cell motility	EF_HAND, EF_HAND_2, efhand	MLR5_FELCA Superfast myosin regulatory light chain 2 (MYLC2)	130
208-209	CT7322	CG2222 unknown		none	
210-211	CT7705	CG2309 ERK7 protein kinase, protein serine/threonine kinase		YPC2_CAEEL Putative serine/threonine-protein kinase C05D10.2 in chromosome III	392
212-213	CT8341	CG2520 lap lap, chaperone	ENTH	(AF182339) clathrin assembly protein AP180 [Loligo pealei]	502
214-215	CT9021	CG2666 CS-1 CS-1, enzyme/chitin synthase		(AF221067) chitin synthase 1 [Lucilia cuprina]	2770
216-217	CT9593	CG2829 BcDNA:GH07910 protein kinase, protein serine/threonine kinase	NLS_BP, PFKB_KINASES_1, PROTEIN_KINASE_ATP, PROTEIN_KINASE_DOM, PROTEIN_KINASE_ST, PRO_RICH, pkinase	(AB004884) PKU-alpha [Homo sapiens]	520
218-219	CT9754	CG2849 Rala Ral, RAS small monomeric GTPase, regulates developmental cell shape changes through the JNK pathway	ATP_GTP_A, PRENYLATION, RASTRNSFRMNG, ras	(XM_035787) similar to Ras-related protein RAL-A [Homo sapiens]	304

220-221	CT9660	CG2829 BcDNA:GH07910 protein kinase, protein serine/threonine kinase	NLS_BP, PFKB_KINASES_1, PROTEIN_KINASE_ATP, PROTEIN_KINASE_DOM, PROTEIN_KINASE_ST, PRO_RICH, pkinase	(AB004884) PKU-alpha [Homo sapiens]	520
222-223	CT6171	CG2968 hydrogen-transporting ATP synthase, coupling factor CF(0), delta-chain		P35434 ATPD_RAT ATP synthase delta chain, mitochondrial precursor	142
224-225	CT10206	CG3034 EG:BACR7A4.6 similar to Surf5b [Homo sapiens]		(Y15172) surfeit protein 5 [Takifugu rubripes]	183
226-227	CT41361	CG3071 EG:25E8.3 involved in retrograde (Golgi to ER) transport which is putatively a component of the coatomer	Trp-Asp (WD) repeats signature protein	T40471 probable Trp-Asp repeat protein - fission yeast	273
228-229	CT9947	CG3071 EG:25E8.3 involved in retrograde (Golgi to ER) transport which is putatively a component of the coatomer	Trp-Asp (WD) repeats signature protein	T40471 probable Trp-Asp repeat protein - fission yeast	273
230-231	CT10723	CG3201 Mlc-c Mlc-c, alkali light chain of non-muscle myosin-II, cytoskeleton organization and biogenesis	EF_HAND, EF_HAND_2, efhand	Homo sapiens 'MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM) (LC17B) (LC' SWP:P24572	
232-233	CT11063	CG3313 transcription factor	NLS_BP, WD40, WD40_REGION	(AB067479) KIAA1892 protein [Homo sapiens]	293
234-235	CT11487	CG3415 estradiol 17 beta-dehydrogenase	ADH_SHORT, GDHRDH, THIOL_PROTEASE_HIS, adh short	(NM_000414) hydroxysteroid (17-beta) dehydrogenase 4 [Homo sapiens]	613
236-237	CT11597	CG3446 unknown		(AJ316011) mitochondrial NADH:ubiquinone oxidoreductase B16.6	78.6
238-239	CT11623	CG3455 Rpt4 Rpt4, endopeptidase, multicatalytic endopeptidase regulator, multicatalytic endopeptidase, proteasome ATPase		Manduca sex '26S proteasome regulatory ATPase subunit 10b (S10b)' EMBL:AJ223384	
240-241	CT11966	CG3560 anon-EST:Posey167 NADH dehydrogenase		1BCC F Chain F, Cytochrome Bc1 Complex From Chicken	150
242-243	CT12417	CG3703 EG:BACR7A4.15 cytoskeleton organization and biogenesis		(NM_075735) T19D7.4.p [Caenorhabditis elegans]	251

244-245	CT12443	CG3715 Shc dShc, SHC-adaptor protein, protein kinase putatively involved in cell growth and maintenance		S25776 transforming protein (SHC) - human	267
246-247	CT12517	CG3747 Eaat1 Eaat1, glutamate transporter, Excitatory amino acid transporter 1	plasma membrane	(AF330257) glutamate transporter [Mus musculus]	402
248-249	CT12871	CG3861 citrate (SI)-synthase	CITRATE_SYNTHASE, CITRATESYNTHASE, citrate_synt	P00889 CISY_PIG CITRATE SYNTHASE, MITOCHONDRIAL PRECURSOR	674
250-251	CT12909	CG3874 nucleotide-sugar transporter-like		(NM_015139) UDP-glucuronic acid/UDP-N-acetylgalactosamine dual	361
252-253	CT13223	CG3981 Unc-76 Dunc-76, signal transducer involved in axon cargo transport		(NM_005102) zygin 2; fasciculation and elongation protein zeta 2;	197
254-255	CT4722	CG4013 Smr Smrter SMRT-related ecdysone receptor-interacting factor SANT domain protein, transcription corepressor	ANTIFREEZE1, myb_DNA-binding	NCR2_MOUSE NUCLEAR RECEPTOR CO-REPRESSOR 2 (N-COR2) (SILENCING MEDIATOR OF	275
256-257	CT13458	CG4094 fumarate hydratase, enzyme involved in main pathways of carbohydrate metabolism	DCRYSTALLIN, FUMARATE_LYASES, FUMARATELYASE, lyase_1	(NM_017005) fumarate hydratase [Rattus norvegicus]	512
258-259	CT13690	CG4129 BcDNA:LD21623 unknown		(XM_043094) KIAA0061 protein [Homo sapiens]	325
260-261	CT5938	CG4147 Hsc70-3 Hsc70-3, Heat shock protein cognate 3, involved in stress response	ER_TARGET, HEATSHOCK70, HSP70, HSP70_1, HSP70_2, HSP70_3	(AB016836) heat shock 70 kD protein cognate [Bombyx mori]	1159
262-263	CT13852	CG4202 Sas10 Sas10		(NM_023054) disrupter of silencing SAS10 [Mus musculus]	259
264-265	CT14019	CG4300 spermidine synthase	SAM_BIND	(AJ009865) spermine synthase [Takifugu rubripes]	276
266-267	CT14119	CG4300 spermidine synthase	SAM_BIND	(AJ009865) spermine synthase [Takifugu rubripes]	276
268-269	CT13914	CG4317 Mipp2 Mipp2, multiple inositol-polyphosphate phosphatase 2	CYTOCHROME_B_QO	Mus musculus 'multiple inositol polyphosphate phosphatase' <u>EMBL:AF046908</u>	
270-271	CT14464	CG4453 transporter, an endopeptidase involved in behavior which is a component of the nucleus	ZF_RANBP, zf-RanBP	14578 nucleoporin Nup153 homolog - African clawed frog (fragment)	300

272- 273	CT14586	CG4481 Glu-RIB ion channel - alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate selective glutamate receptor; ionotropic glutamate receptor	ANF_receptor, CHANNEL_PORE_K, NLS_BP, SBP_GLUR, lig_chan	<u>Mus musculus 'glutamate receptor channel a3 subunit'</u> <u>EMBL:AB022342</u>	
274- 275	CT14874	CG4590 inx2 inx2, neurotransmitter transporter, Dm-inx pas related protein 33	Innexin	<u>Schistocerca americana 'innexin-2'</u> EMBL:115854_1	
276- 277	CT15952	CG4974 dally NOT cell adhesion molecule; heparin sulfate proteoglycan; Dally	Glypican	(NM_004466) glypican 5 [Homo sapiens]	186
278- 279	CT16489	CG5147 unknown		none	
280- 281	CT16663	CG5208 BcDNA:LD27979 unknown		none	
282- 283	CT17394	CG5485 high affinity sulfate permease, sulfate transporter		(AF349043) sulfate anion transporter-1 [Mus musculus]	340
284- 285	CT17382	CG5486 Ubp64E Ubiquitin-specific protease 64E		(NM_063285) ubiquitin carboxyl-terminal hydrolase [Caenorhabditis]	358
286- 287	CT17448	CG5505 endopeptidase, ubiquitin-specific protease, involved in process of deubiquitylation	UCH-1, UCH-2, UCH_2_1, UCH_2_2, UCH_2_3	(XM_027039) KIAA1453 protein [Homo sapiens]	254
288- 289	CT17938	CG5684 non-specific RNA polymerase II transcription factor		Q9UTV1 CNO7_HUMAN CCR4-NOT transcription complex, subunit 7 (CCR4-associated factor)	376
290- 291	CT17971	CG5722 NPC1 dmNPC1, transmembrane receptor	5TM_BOX, NLS_BP	(NM_000271) Niemann-Pick disease, type C1 [Homo sapiens]	1061
292- 293	CT18192	CG5797 cytoskeletal binding protein	PRO_RICH	(AB051482) KIAA1695 protein [Homo sapiens]	541
294- 295	CT18619	CG5939 Prm Para, Paramyosin, structural protein of muscle, motor	NLS_BP	(AF317670) paramyosin [Sarcoptes scabiei]	989
296- 297	CT18969	CG6058 Ald fructose-bisphosphate aldolase, involved in process of glycolysis	ALDOLASE_CLASS_I, NLS_BP, glycolytic_enzy	<u>Mus musculus Aldo1</u> <u>MGI:87994</u>	
298- 299	CT19788	CG6335 histidine--tRNA ligase	AA_TRNA_LIGASE_II_1, AA_TRNA_LIGASE_II_2, WHEP-TRS, tRNA-synt_2b	(NM_008214) histidyl tRNA synthetase [Mus musculus]	641

300-301	CT19850	CG6367 serine-type endopeptidase		(AF053921) trypsin-like serine protease [Ctenocephalides felis]	163
302-303	CT19962	CG6400 unknown	BROMODOMAIN, BROMODOMAIN_2, GPROTEINBRPT, NLS_BP, WD40, WD40_REGION, WD_REPEATS, bromodomain	Q9NSI6[WDR9_HUMAN WD-REPEAT PROTEIN 9]	916
304-305	CT20122	CG6470 unknown	ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	none	
306-307	CT20269	CG6513 signal transduction		(NM_019561) endosulfine alpha; alpha-endosulfine [Mus musculus]	91.3
308-309	CT21021	CG6774 tracheal gasfilling mutant		(NM_023037) hypothetical protein CG003 [Homo sapiens]	1006
310-311	CT21292	CG6874 unknown		none	
312-313	CT43217	CG6928 Sulfate transporter	Sulfate_transp		
314-315	CT21476	CG6930 unknown	NLS_BP, ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	Caenorhabditis elegans 'contains strong similarity to a C2H2-type zinc finger' EMBL:AF000194	
316-317	CT21525	CG6946 RNA binding	RBD, rrm	Rattus norvegicus 'ribonucleoprotein F' EMBL:AB022209	
318-319	CT21704	CG7014 structural protein of ribosome, Process protein biosynthesis	RIBOSOMAL_S7, Ribosomal_S7	(NM_001009) ribosomal protein S5; 40S ribosomal protein S5 [Homo]	347
320-321	CT22195	CG7187 DNA binding		(AY026310) single stranded DNA binding protein-1 [Homo sapiens]	351
322-323	CT22253	CG7215 ubiquitin	UBIQUITIN_2, ubiquitin	P21126[UBLG_MOUSE Ubiquitin-like protein GDX (Ubiquitin-like protein 4)]	75.5
324-325	CT22861	CG7434 RpL22 ribosomal protein L22	ANTIFREEZEI	(AF400188) ribosomal protein L22 [Spodoptera frugiperda]	165
326-327	CT23083	CG7552 unknown	ATP_GTP_A, WW_DOMAIN_1, WW_DOMAIN_2, WW_rsp5 WWP	Homo sapiens '65 KD YES-ASSOCIATED PROTEIN (YAP65)' SWP:P46937	
328-329	CT23596	CG7757 similarity to U4/U6-associated RNA splicing factor	NLS_BP	(NM_004698) U4/U6-associated RNA splicing factor [Homo sapiens]	520

330-331	CT23626	CG7770 cochaperonin in process of 'de novo' protein folding		(NM_010385) H2-K region expressed gene 2 [Mus musculus]	106
332-333	CT23882	CG7901 PP2A-B' protein phosphatase, protein phosphatase type 2A regulator	ANTIFREEZE1	Mus musculus 'protein phosphatase 2A B'a3 regulatory subunit' EMBL:U37353	
334-335	CT41698	CG7958 unknown		(AB033050) KIAA1224 protein [Homo sapiens]	427
336-337	CT23982	CG7958 unknown		(AB033050) KIAA1224 protein [Homo sapiens]	427
338-339	CT23998	CG7983 guanylate kinase	PRO_RICH	(AF411837) transcription repressor p66 [Mus musculus]	214
340-341	CT24094	CG8031 unknown		(BC013819) CGI-27 protein [Mus musculus]	394
342-343	CT24122	CG8037 ELL, DNA-directed RNA polymerase III;		Gallus gallus 'OCCLUDIN' SWP:Q91049	
344-345	CT24346	CG8148 timeout timeout		(NM_003920) timeless (Drosophila) homolog [Homo sapiens]	149
346-347	CT24393	CG8189 ATPsyn-b ATPsyn-b Fo-ATP synthase subunit b	Acetyltransf	(AF187862) ATP synthase subunit B [Xenopus laevis]	213
348-349	CT24437	CG8231 T-complex protein 1, zeta-subunit, chaperone	CHAPERONIN60, TCOMPLEXTCP1, TCP1_1, TCP1_2, TCP1_3, cpn60_TCP1	O77622 TCPZ_RABIT T-COMPLEX PROTEIN 1, ZETA SUBUNIT (TCP-1-ZETA) (CCT-ZETA)	754
350-351	CT18257	CG8322 ATPCL ATP-citrate (pro-S)-lyase	SUCCINYL_COA_LIG_1, SUCCINYL_COA_LIG_2, SUCCINYL_COA_LIG_3, ligase-CoA	(U18197) ATP:citrate lyase [Homo sapiens]	1555
352-353	CT24731	CG8439 Cct5 Cct5, T-complex Chaperonin 5, tracheal gasfilling mutant		(XM_052313) chaperonin containing TCP1, subunit 5 (epsilon) [Homo]	791
354-355	CT24823	CG8484 Transcription factor	ZINC_FINGER_C2H2, ZINC_FINGER_C2H2_2, zf-C2H2	(NM_058230) zinc finger protein 354B [Homo sapiens]	167
356-357	CT25072	CG8655 CDC receptor signaling protein serine/threonine kinase	AA_TRNA_LIGASE_II_2, PROTEIN_KINASE_DOM, PROTEIN_KINASE_ST, pkinase	(AF005209) HsCdc7 [Homo sapiens]	216
358-359	CT25274	CG8759 Nacalpa; NAC protein alpha subunit, component of the nascent polypeptide-associated complex		Homo sapiens &agr PIR:S49326	



360-361	CT25472	CG8870 endopeptidase, monophenol monooxygenase activator	ANTENNAPEDIA, CHYMOTRYPSIN, TRYPSIN_CATAL, TRYPSIN_HIS, TRYPSIN_SER, trypsin	<u>Caenorhabditis elegans 'similar to plasminogen and to trypsin-like serine proteases'</u> <u>EMBL:U29380</u>	
362-363	CT25624	CG8922 RpS5 Ribosomal protein S5	RIBOSOMAL_S7, Ribosomal_S7	(Y12431) 5S ribosomal protein [Mus musculus]	353
364-365	CT8969	CG9165 enzyme, hydroxymethylbilane synthase	PORPHBDMNASE, Porphobil_deam	P08397 HEM3_HUMAN PORPHOBILINOGEN DEAMINASE (HYDROXYMETHYLBILANE SYNTHASE) (HMBS)	287
366-367	CT27084	CG9591 unknown		(XM_043261) KIAA1698 protein [Homo sapiens]	116
368-369	CT27543	CG9748 cap Belle, ATP dependent helicase		1705301A ATP dependent RNA helicase [Xenopus laevis]	723
370-371	CT27750	CG9821 unknown		none	
372-373	CT27796	CG9901 Arp14D Actin-related protein 14D, arp2	ACTIN, ACTINS_ACT_LIKE, actin	P53488 ARP2_CHICK ACTIN-LIKE PROTEIN 2 (ACTIN-LIKE PROTEIN ACTL)	678
374-375	CT27906	CG9910 katanin-80 katanin 80, microtubule severing which is a component of the katanin		(AF052433) katanin p80 subunit [Strongylocentrotus purpuratus]	231
376-377	CT27940	CG9924 transcription factor	BTB, MATH	(NM_003563) speckle-type POZ protein [Homo sapiens]	599
378-379	CT27993	CG9946 eIF-2alpha; Eukaryotic initiation factor 2A; translation initiation factor	NLS_BP, S1	(NM_131800) eIF2 alpha subunit [Danio rerio]	376
380-381	CT20536	CG6606 unknown	ATPASE_ALPHA_BETA, ATP_GTP_A, C2, NLS_BP, RECEPTOR_CYTOKINES_2	(AB020664) KIAA0857 protein [Homo sapiens]	122

## DEFINITIONS

For clarity, certain terms used in the specification are defined and used as follows:

"Associated with / operatively linked" refer to two nucleic acid sequences that are related physically or functionally. For example, a promoter or regulatory DNA sequence is said to be "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

A "chimeric construct" is a recombinant nucleic acid sequence in which a promoter or regulatory nucleic acid sequence is operatively linked to, or associated with, a nucleic acid sequence that codes for an mRNA or which is expressed as a protein, such that the regulatory nucleic acid sequence is able to regulate transcription or expression of the associated nucleic acid sequence. The regulatory nucleic acid sequence of the chimeric construct is not normally operatively linked to the associated nucleic acid sequence as found in nature.

Co-factor: natural reactant, such as an organic molecule or a metal ion, required in an enzyme-catalyzed reaction. A co-factor is e.g. NAD(P), riboflavin (including FAD and FMN), folate, molybdopterin, thiamin, biotin, lipoic acid, pantothenic acid and coenzyme A, S-adenosylmethionine, pyridoxal phosphate, ubiquinone, menaquinone. Optionally, a co-factor can be regenerated and reused.

A "coding sequence" is a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Preferably the RNA is then translated in an organism to produce a protein.

Complementary: "complementary" refers to two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between the complementary base residues in the antiparallel nucleotide sequences.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acid sequences that encode identical or essentially identical amino acid sequences, or where the nucleic acid sequence does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance the codons CGT, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded protein. Such nucleic acid variations are "silent variations" which are one species of "conservatively modified variations." Every nucleic acid sequence described herein which encodes a protein also describes every possible silent variation, except where otherwise noted. One of skill will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon for methionine) can be modified to yield a

functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a protein is implicit in each described sequence.

Furthermore, one of skill will recognize that individual substitutions deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations," where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following five groups each contain amino acids that are conservative substitutions for one another: Aliphatic: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I); Aromatic: Phenylalanine (F), Tyrosine (Y), Tryptophan (W); Sulfur-containing: Methionine (M), Cysteine (C); Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E), Asparagine (N), Glutamine (Q). *See also*, Creighton (1984) *Proteins*, W.H. Freeman and Company. In addition, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are also "conservatively modified variations."

**DNA Shuffling:** DNA shuffling is a method to rapidly, easily and efficiently introduce mutations or rearrangements, preferably randomly, in a DNA molecule or to generate exchanges of DNA sequences between two or more DNA molecules, preferably randomly. The DNA molecule resulting from DNA shuffling is a shuffled DNA molecule that is a non-naturally occurring DNA molecule derived from at least one template DNA molecule. The shuffled DNA encodes an enzyme modified with respect to the enzyme encoded by the template DNA, and preferably has an altered biological activity with respect to the enzyme encoded by the template DNA.

**Enzyme/Protein Activity:** means herein the ability of an enzyme (or protein) to catalyze the conversion of a substrate into a product. A substrate for the enzyme comprises the natural substrate of the enzyme but also comprises analogues of the natural substrate, which can also be converted, by the enzyme into a product or into an analogue of a product. The activity of the enzyme is measured for example by determining the amount of product in the reaction after a certain period of time, or by determining the amount of substrate remaining in the reaction mixture after a certain period of time. The activity of the enzyme is also measured by

determining the amount of an unused co-factor of the reaction remaining in the reaction mixture after a certain period of time or by determining the amount of used co-factor in the reaction mixture after a certain period of time. The activity of the enzyme is also measured by determining the amount of a donor of free energy or energy-rich molecule (e.g. ATP, phosphoenolpyruvate, acetyl phosphate or phosphocreatine) remaining in the reaction mixture after a certain period of time or by determining the amount of a used donor of free energy or energy-rich molecule (e.g. ADP, pyruvate, acetate or creatine) in the reaction mixture after a certain period of time.

Essential: an "essential" *Drosophila melanogaster* nucleotide sequence is a nucleotide sequence encoding a protein such as e.g. a biosynthetic enzyme, receptor, signal transduction protein, structural gene product, or transport protein that is essential to the growth or survival of the insect.

Expression Cassette: "Expression cassette" as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operatively linked to the nucleotide sequence of interest which is operatively linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA, in the sense or antisense direction. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host, i.e., the particular DNA sequence of the expression cassette does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, such as an insect, the promoter can also be specific to a particular tissue or organ or stage of development.

**Gene:** the term "gene" is used broadly to refer to any segment of DNA associated with a biological function. Thus, genes include coding sequences and/or the regulatory sequences required for their expression. Genes also include nonexpressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

**Heterologous/exogenous:** The terms "heterologous" and "exogenous" when used herein to refer to a nucleic acid sequence (e.g. a DNA sequence) or a gene, refer to a sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified through, for example, the use of DNA shuffling. The terms also include non-naturally occurring multiple copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is not ordinarily found. Exogenous DNA segments are expressed to yield exogenous polypeptides.

A "homologous" nucleic acid (e.g. DNA) sequence is a nucleic acid (e.g. DNA) sequence naturally associated with a host cell into which it is introduced.

The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

**Inhibitor:** a chemical substance that inactivates the enzymatic activity of an enzyme (or protein) of interest. The term "insecticide" is used herein to define an inhibitor when applied to an insect at any stage of development.

**Insecticide:** a chemical substance used to kill or inhibit the growth or viability of insects at any stage of development.

**Interaction:** quality or state of mutual action such that the effectiveness or toxicity of one protein or compound on another protein is inhibitory (antagonists) or enhancing (agonists).

A nucleic acid sequence is "isocoding with" a reference nucleic acid sequence when the nucleic acid sequence encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the reference nucleic acid sequence.

An "isolated" nucleic acid molecule or an isolated enzyme is a nucleic acid molecule or enzyme that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or enzyme may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell.

**Mature Protein:** protein that is normally targeted to a cellular organelle and from which the transit peptide has been removed.

**Minimal Promoter:** promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription.

**Modified Enzyme Activity:** enzyme activity different from that which naturally occurs in an insect (i.e. enzyme activity that occurs naturally in the absence of direct or indirect manipulation of such activity by man), which is tolerant to inhibitors that inhibit the naturally occurring enzyme activity.

**Native:** refers to a gene that is present in the genome of an untransformed insect cell.

**Naturally occurring:** the term "naturally occurring" is used to describe an object that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

**Nucleic acid:** the term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which

the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19: 5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260: 2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8: 91-98 (1994)). The terms "nucleic acid" or "nucleic acid sequence" may also be used interchangeably with gene, cDNA, and mRNA encoded by a gene.

"ORF" means open reading frame.

**Purified:** the term "purified," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein which is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

Two nucleic acids are "recombined" when sequences from each of the two nucleic acids are combined in a progeny nucleic acid. Two sequences are "directly" recombined when both of the nucleic acids are substrates for recombination. Two sequences are "indirectly recombined" when the sequences are recombined using an intermediate such as a cross-over oligonucleotide. For indirect recombination, no more than one of the sequences is an actual substrate for recombination, and in some cases, neither sequence is a substrate for recombination.

"Regulatory elements" refer to sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements comprise a promoter operatively linked to the nucleotide sequence of interest and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

**Significant Increase:** an increase in enzymatic activity that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater of the activity of the wild-type enzyme in the presence of the inhibitor, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

**Substantially identical:** the phrase "substantially identical," in the context of two nucleic acid or protein sequences, refers to two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90, even more preferably 95%, and most preferably at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In an especially preferred embodiment, the sequences are substantially identical over the entire length of the coding regions. Furthermore, substantially identical nucleic acid or protein sequences perform substantially the same function.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally*, Ausubel *et al.*, *infra*).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information on the world wide web at [ncbi.nlm.nih.gov/](http://ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some



positive-valued threshold score  $T$  when aligned with a word of the same length in a database sequence.  $T$  is referred to as the neighborhood word score threshold (Altschul *et al.*, 1990). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters  $M$  (reward score for a pair of matching residues; always  $> 0$ ) and  $N$  (penalty score for mismatching residues; always  $< 0$ ). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity  $X$  from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters  $W$ ,  $T$ , and  $X$  determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength ( $W$ ) of 11, an expectation ( $E$ ) of 10, a cutoff of 100,  $M=5$ ,  $N=-4$ , and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength ( $W$ ) of 3, an expectation ( $E$ ) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89: 10915 (1989)).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90: 5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ( $P(N)$ ), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex

mixture (*e.g.*, total cellular) DNA or RNA. "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes* part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize to its target subsequence, but to no other sequences.

The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the  $T_m$  for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 5M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook, *infra*, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. For short probes (*e.g.*, about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be

achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a reference nucleotide sequence preferably hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

A further indication that two nucleic acid sequences or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with, or specifically binds to, the protein encoded by the second nucleic acid. Thus, a protein is typically substantially identical to a second protein, for example, where the two proteins differ only by conservative substitutions.

The phrase "specifically (or selectively) binds to an antibody," or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the protein with the amino acid sequence encoded by any of the nucleic acid sequences

of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with other proteins except for polymorphic variants. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York "Harlow and Lane"), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

A "subsequence" refers to a sequence of nucleic acids or amino acids that comprise a part of a longer sequence of nucleic acids or amino acids (e.g., protein) respectively.

"Synthetic" refers to a nucleotide sequence comprising structural characters that are not present in the natural sequence. For example, an artificial sequence that resembles more closely the G+C content and the normal codon distribution of dicot and/or monocot genes is said to be synthetic.

**Substrate:** a substrate is the molecule that an enzyme naturally recognizes and converts to a product in the biochemical pathway in which the enzyme naturally carries out its function, or is a modified version of the molecule, which is also recognized by the enzyme and is converted by the enzyme to a product in an enzymatic reaction similar to the naturally-occurring reaction.

**Target gene:** A "target gene" is any gene in an insect cell. For example, a target gene is a gene of known function or is a gene whose function is unknown, but whose total or partial nucleotide sequence is known. Alternatively, the function of a target gene and its nucleotide sequence are both unknown. A target gene is a native gene of the insect cell or is a heterologous gene that had previously been introduced into the insect cell or a parent cell of said insect cell, for example by genetic transformation. A heterologous target gene is stably integrated in the genome of the insect cell or is present in the insect cell as an extrachromosomal molecule, e.g. as an autonomously replicating extrachromosomal molecule.

**Transformation:** a process for introducing heterologous DNA into a cell, tissue, or insect. Transformed cells, tissues, or insects are understood to encompass not only the end product of a

transformation process, but also transgenic progeny thereof.

“Transformed,” “transgenic,” and “recombinant” refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A “non-transformed,” “non-transgenic,” or “non-recombinant” host refers to a wild-type organism, e.g., a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

Viability: “viability” as used herein refers to a fitness parameter of an insect. Insects are assayed for their homozygous performance of *Drosophila* development, indicating which proteins are indispensable to maintain life in *Drosophila*.

#### DETAILED DESCRIPTION OF THE INVENTION

##### I. Identification Of Essential *Drosophila melanogaster* Nucleotide Sequences Using Transposable Element Insertion Mutagenesis

As shown in Table 2 and the examples below, the identification of novel nucleotide sequences, as well as the essentiality of the nucleotide sequences for normal insect viability, have been demonstrated in *Drosophila* using P-element transposable insertion mutagenesis. Having established the essentiality of the function of the encoded proteins in *Drosophila* and having identified the nucleotide sequences encoding these essential proteins, the inventors thereby provide an important and sought-after tool for new insecticide development.

A lethal phenotype caused by insertion of a P-element indicates that the affected nucleotide sequence codes for an essential protein in the insect. The characterization of the insertion site using flanking sequence DNA is needed to associate an individual lethal line with specific nucleotide sequences. Genomic DNA adjacent to the 5’ and/or 3’ end of the P-element from the insertion line is generated using inverse PCR.

Table 2 Method of validation of nucleic acid sequences as essential

SEQ ID NO	validation method
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14	dsRNA and p-element disruption
16	p-element disruption
18	p-element disruption
20	p-element disruption
22	p-element disruption
24	p-element disruption
26	p-element disruption
28	p-element disruption
30	dsRNA
32	p-element disruption
34	p-element disruption
36	p-element disruption
38	p-element disruption
40	p-element disruption
42	dsRNA
44	p-element disruption
46	p-element disruption
48	p-element disruption
50	p-element disruption
52	DsRNA
54	p-element disruption
56	p-element disruption
58	p-element disruption
60	p-element disruption
62	p-element disruption
64	p-element disruption
66	p-element disruption
68	DsRNA
70	DsRNA
72	DsRNA
74	p-element disruption
76	p-element disruption
78	p-element disruption
80	p-element disruption
82	p-element disruption
84	p-element disruption
86	DsRNA
88	p-element disruption
90	p-element disruption
92	p-element disruption
94	p-element disruption
96	p-element disruption
98	p-element disruption
100	p-element disruption
102	p-element disruption
104	p-element disruption
106	dsRNA and p-element disruption
108	p-element disruption

110	DsRNA
112	p-element disruption
114	DsRNA
116	p-element disruption
118	p-element disruption
120	p-element disruption
122	p-element disruption
124	p-element disruption
126	p-element disruption
128	p-element disruption
130	p-element disruption
132	p-element disruption
134	p-element disruption
136	p-element disruption
138	p-element disruption
140	p-element disruption
142	p-element disruption
144	p-element disruption
146	p-element disruption
148	p-element disruption
150	p-element disruption
152	p-element disruption
154	p-element disruption
156	p-element disruption
158	p-element disruption
160	DsRNA
162	p-element disruption
164	p-element disruption
166	p-element disruption
168	p-element disruption
170	p-element disruption
172	p-element disruption
174	p-element disruption
176	p-element disruption
178	p-element disruption
180	p-element disruption
182	p-element disruption
184	p-element disruption
186	p-element disruption
188	p-element disruption
190	p-element disruption
192	p-element disruption
194	DsRNA
196	p-element disruption
198	p-element disruption
200	p-element disruption
202	p-element disruption
204	DsRNA

206	p-element disruption
208	p-element disruption
210	p-element disruption
212	p-element disruption
214	DsRNA
216	p-element disruption
218	p-element disruption
220	p-element disruption
222	DsRNA
224	p-element disruption
226	p-element disruption
227	p-element disruption
228	p-element disruption
230	p-element disruption
232	p-element disruption
234	p-element disruption
236	p-element disruption
238	p-element disruption
240	p-element disruption
242	p-element disruption
244	DsRNA
246	p-element disruption
248	p-element disruption
250	p-element disruption
252	p-element disruption
254	p-element disruption
256	p-element disruption
258	p-element disruption
260	p-element disruption
262	p-element disruption
264	p-element disruption
266	p-element disruption
268	p-element disruption
270	p-element disruption
272	dsRNA and p-element disruption
274	p-element disruption
276	p-element disruption
278	p-element disruption
280	p-element disruption
282	p-element disruption
284	p-element disruption
286	DsRNA
288	DsRNA
290	p-element disruption
292	p-element disruption
294	p-element disruption
296	DsRNA
298	DsRNA



300	p-element disruption
302	p-element disruption
304	p-element disruption
306	p-element disruption
308	p-element disruption
310	p-element disruption
312	p-element disruption
314	p-element disruption
316	p-element disruption
318	p-element disruption
320	p-element disruption
322	p-element disruption
324	p-element disruption
326	p-element disruption
328	p-element disruption
330	p-element disruption
332	p-element disruption
334	p-element disruption
336	p-element disruption
338	p-element disruption
340	p-element disruption
342	dsRNA and p-element disruption
344	DsRNA
346	p-element disruption
348	dsRNA and p-element disruption
350	DsRNA
352	dsRNA and p-element disruption
354	p-element disruption
356	p-element disruption
358	dsRNA and p-element disruption
360	p-element disruption
362	p-element disruption
364	p-element disruption
366	p-element disruption
368	DsRNA
370	p-element disruption
372	p-element disruption
374	p-element disruption
376	p-element disruption
378	p-element disruption
380	p-element disruption

# I. Determining The Complete Coding Sequences Of The Essential *Drosophila* Nucleotide Sequences

The essential *Drosophila* nucleotide sequences are identified by isolating nucleotide sequences flanking the P-element insertion and aligning that sequence with genomic *Drosophila* sequence obtained from the Celera *Drosophila* database. The protein prediction for each genomic region is obtained by use of an exon algorithm program such as GeneMark. All exon algorithm programs currently used for prediction of proteins are susceptible to inaccuracies, including incomplete predictions of coding sequences, missing alternative splice variants, combining of nearby exons of adjacent genes, and mistranslation at intron-exon borders. The prediction of a complete coding sequence can be confirmed by several methods including polymerase chain reaction (PCR) amplification using the 5' and 3' sequence to verify the message, reverse transcription PCR (rtPCR) using an oligonucleotide internal sequence to identify the 5' and/or 3' end, and screening of cDNA libraries from insect tissues with probes made from a particular sequence to isolate a true full-length clone. To confirm that the message size is accurate, a Northern blot can be hybridized with a probe from the nucleotide sequence. In addition, matches to the *Drosophila* EST database helps to confirm existence of message and gives information about the temporal and spatial pattern of expression. Mutation-causing P elements are known to preferentially cluster in the 5' region of affected genes (Spradling *et al.*, *Proc. Natl. Acad. Sci. USA* 92: 10824-10830 (1995)), a tendency that increases the chance of recovering overlaps between short flanking sequences and 5' ESTs. The present invention therefore provides a number of essential nucleotide sequences as well as the amino acid sequences encoded thereby. cDNA clone sequences are set forth in even numbered SEQ ID NOs:14-380. The corresponding encoded amino acid sequences are set forth in odd numbered SEQ ID NOs:15-381.

The isolated gene sequences disclosed herein may be manipulated according to standard genetic engineering techniques to suit any desired purpose. For example, an entire *Drosophila* gene sequence or portions thereof may be used as a probe capable of specifically hybridizing to coding sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include, e.g. sequences that are unique among insect nucleotide sequences for a particular protein of interest and are at least 10 nucleotides in length, preferably at least 20 nucleotides in length, and most preferably at least 50 nucleotides in length. Such probes are used to amplify and analyze related nucleotide sequences from a chosen organism via

PCR. This technique is useful to isolate additional insect nucleotide sequences from a desired organism or as a diagnostic assay to determine the presence of particular nucleotide sequences in an organism. This technique also is used to detect the presence of altered nucleotide sequences associated with a particular condition of interest such as insecticide tolerance, poor health, etc.

Gene-specific hybridization probes also are used to quantify levels of a particular gene mRNA in an insect using standard techniques such as Northern blot analysis. This technique is useful as a diagnostic assay to detect altered levels of gene expression that are associated with particular conditions such as enhanced tolerance to insecticides that target a particular gene.

#### I.A. Identification of Essential *Drosophila melanogaster* Nucleotide Sequences using RNAi

RNA-mediated interference (RNAi) is a recently discovered method to determine gene function in a number of organisms, wherein double-stranded RNA (dsRNA) directs gene-specific, post-transcriptional silencing. See, e.g., Kuwabara & Olson (2000) *Parasitol Today* 16(8):347-349; Bass (2000) *Cell* 101(3):235-238; Hunter (2000) *Curr Biol* 10(4):R137-140; Boshier & Labouesse (2000) *Nat Cell Biol* 2(2):E31-36; Sharp (1999) *Genes Dev* 13(2):139-141. The double-stranded RNA molecule can be synthesized in vitro and then introduced into the organism by injection or other methods. Alternatively, a heritable transgene exhibiting dyad symmetry can provide a transcript that folds as a hairpin structure. Methods for examining gene functions using dsRNAi in *Drosophila* are disclosed in Example 4a and further in Kennerdell & Carthew (2000) *Nat Biotech* 18(8):896-898; Lam & Thummel (2000) *Curr Biol* 10(16):957-963; Misquitta & Paterson (1999) *Proc Natl Acad Sci USA* 96 (4):1451-1456.

The present invention describes RNA-mediated interference of sequences listed in Table 2 and Table 6. Double-stranded RNA complementary to each sequence was synthesized in vitro and injected into early *Drosophila* embryos, as described in Example 4a. Development of injected embryos was assessed by scoring: (a) morphological criteria using a light microscope (Campos-Ortega & Hartenstein (1985) *The Embryonic Development of Drosophila melanogaster*, Springer-Verlag, Berlin), (b) embryo hatching to become a larvae, (c) puparium formation, and (d) eclosion of the pupae as an adult fly, as indicated in Table 6 herein below. Buffer-injected embryos were injected and monitored in parallel as a control. The percentage of embryos

injected with dsRNA that survive to the adult stage is depicted in set forth in Table 6.

Essential genes were identified as those resulting in a percent viable adults below 38% when disrupted by RNAi. This threshold was determined by comparison to multiple buffer-injected controls.

## II. Recombinant Production Of Protein And Uses Thereof

For recombinant production of a protein of the invention in a host organism, a nucleotide sequence encoding the protein is inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. The choice of the specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequence, and enhancer appropriate for the chosen host is within the level of the skill of the routineer in the art. The resultant molecule, containing the individual elements linking in the proper reading frame, is inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli*, yeast, and insect cells (see, e.g., Lucknow and Summers, *Bio/Technol.* 6:47 (1988)). Additional suitable expression vectors are baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV). A preferred baculovirus/insect system is PVL1392(3) used to transfect *Spodoptera frugiperda* SF9 cells (ATCC) in the presence of linear *Autographica californica* baculovirus DNA (Phramingen, San Diego, CA). The resulting virus is used to infect HighFive *Tricoplusia ni* cells (Invitrogen, La Jolla, CA).

Recombinantly produced proteins are isolated and purified using a variety of standard techniques. The actual techniques used vary depending upon the host organism used, whether the protein is designed for secretion, and other such factors. Such techniques are well known to the skilled artisan (see, e.g. chapter 16 of Ausubel, F. et al., "Current Protocols in Molecular Biology", pub. by John Wiley & Sons, Inc. (1994)).

## IV. Assays For Characterizing The Proteins

Recombinantly produced proteins are useful for a variety of purposes. For example, they can be used in in vitro assays to screen known insecticidal chemicals whose target has not been identified to determine if they inhibit protein activity. Such in vitro assays may also be used as more general screens to identify chemicals that inhibit such protein activity and that are therefore novel insecticide candidates. Recombinantly produced proteins may also be used to elucidate the complex structure of these molecules and to further characterize their association with known inhibitors in order to rationally design new inhibitory insecticides. Alternatively, the recombinant protein can be used to isolate antibodies or peptides that modulate the activity and are useful in transgenic solutions.

#### V. *In vivo* Inhibitor Assay: Discovery of Small Molecule Ligands That Interact with Proteins Of Unknown Function.

Having identified a protein as a potential insecticide target based on its essentiality for insect viability, a next step is to develop an assay that allows screening large numbers of chemicals to determine which ones interact with the protein. Although it is straightforward to develop assays for proteins of known function, developing assays with proteins of unknown functions can be more difficult.

To address this issue, novel technologies are used that can detect interactions between a protein and a ligand without knowing the biological function of the protein. A short description of three methods is presented, including fluorescence correlation spectroscopy, surface-enhanced laser desorption/ionization, and biacore technologies. In addition to those described here, there are additional methods that are currently being developed that are also amenable to automated, large-scale screening.

Fluorescence Correlation Spectroscopy (FCS) theory was developed in 1972 but it is only in recent years that the technology to perform FCS became available (Madge et al. (1972) *Phys. Rev. Lett.*, 29: 705-708; Maiti et al. (1997) *Proc. Natl. Acad. Sci. USA*, 94: 11753-11757). FCS measures the average diffusion rate of a fluorescent molecule within a small sample volume. The sample size can be as low as  $10^3$  fluorescent molecules and the sample volume as low as the cytoplasm of a single bacterium. The diffusion rate is a function of the mass of the molecule and decreases as the mass increases. FCS can therefore be applied to protein-ligand interaction

analysis by measuring the change in mass and therefore in diffusion rate of a molecule upon binding. In a typical experiment, the target to be analyzed is expressed as a recombinant protein with a sequence tag, such as a poly-histidine sequence, inserted at the N- or C-terminus. The expression takes place in *E. coli*, yeast or insect cells. The protein is purified by chromatography. For example, the poly-histidine tag can be used to bind the expressed protein to a metal chelate column such as Ni<sup>2+</sup> chelated on iminodiacetic acid agarose. The protein is then labeled with a fluorescent tag such as carboxytetramethylrhodamine or BODIPY® (Molecular Probes, Eugene, OR). The protein is then exposed in solution to the potential ligand, and its diffusion rate is determined by FCS using instrumentation available from Carl Zeiss, Inc. (Thornwood, NY). Ligand binding is determined by changes in the diffusion rate of the protein.

Surface-Enhanced Laser Desorption/Ionization (SELDI) was invented by Hutchens and Yip during the late 1980's (Hutchens and Yip (1993) *Rapid Commun. Mass Spectrom.* 7: 576-580). When coupled to a time-of-flight mass spectrometer (TOF), SELDI provides means to rapidly analyze molecules retained on a chip. It can be applied to ligand-protein interaction analysis by covalently binding the target protein on the chip and analyze by MS the small molecules that bind to this protein (Worrall et al. (1998) *Anal. Biochem.* 70: 750-756). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the SELDI chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via, for example, a delivery system able to pipet the ligands in a sequential manner (autosampler). The chip is then submitted to washes of increasing stringency, for example a series of washes with buffer solutions containing an increasing ionic strength. After each wash, the bound material is analyzed by submitting the chip to SELDI-TOF. Ligands that specifically bind the target will be identified by the stringency of the wash needed to elute them.

Biacore relies on changes in the refractive index at the surface layer upon binding of a ligand to a protein immobilized on the layer. In this system, a collection of small ligands is injected sequentially in a 2-5 microlitre cell with the immobilized protein. Binding is detected by surface plasmon resonance (SPR) by recording laser light refracting from the surface. In general, the refractive index change for a given change of mass concentration at the surface layer is

practically the same for all proteins and peptides, allowing a single method to be applicable for any protein (Liedberg et al. (1983) *Sensors Actuators* 4: 299-304; Malmquist (1993) *Nature* 361: 186-187). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the Biacore chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via the delivery system incorporated in the instruments sold by Biacore (Uppsala, Sweden) to pipet the ligands in a sequential manner (autosampler). The SPR signal on the chip is recorded and changes in the refractive index indicate an interaction between the immobilized target and the ligand. Analysis of the signal kinetics on rate and off rate allows the discrimination between non-specific and specific interaction.

The compounds that are active in the methods disclosed herein may be used to combat agricultural pests such as aphids, locusts, spider mites, and boll weavils as well as such insect pests which attack stored grains and against immature stages of insects living on plant tissue. The compounds are also useful as a nematocide for the control of agriculturally important soil nematodes and plant parasites.

## VI. Production of peptides

Phage particles displaying diverse peptide libraries permits rapid library construction, affinity selection, amplification and selection of ligands directed against an essential protein (H.B. Lowman, *Annu. Rev. Biophys. Biomol. Struct.* 26, 401-424 (1997)). Structural analysis of these selectants can provide new information about ligand-target molecule interactions and then in the process also provide a novel molecule that can enable the development of new insecticides based upon these peptides as leads.

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

## EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, *et al.*, *Molecular Cloning*, eds., Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, NY (1989) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987). Well known *Drosophila* molecular genetics techniques can be found, for example, in Robert, D.B., *Drosophila, A Practical Approach* (IRL Press, Washington, DC, 1986).

### Example 1: Identification Of Lethal Lines

Essential nucleotide sequences are identified through the isolation of lethal mutants defective in development. The genetic scheme for mobilization of P-lacW is as performed in Deak *et al.*, *Genetics* 147: 1697-1722 (1997). Additional lethal lines are identified and disclosed in Braun, A., B. Lemaitre, *et al.*, *Genetics* 147: 623-634 (1997); Galloni, M. and B. A. Edgar, *Development* 126: 2365-2375 (1999); Gateff, E., *Int. J. Dev. Biol.* 38(4): 565-590 (1994); Mechler, B. M. J. Biosci., *Bangalore* 19(5): 537-556 (1994); Roch, F., F. Serras, *et al.*, *Mol. Gen. Genet.* 257: 103-112 (1998); Russell, M. A., L. Ostafichuk, *et al.*, *Genome* 41: 7-13 (1998); and in Torok, T., G. Tick, *et al.*, *Genetics* 135: 71-80 (1993), Schaefer *et al.*, 1999.8.12 Personal communication to FlyBase. Furthermore, the BDGP gene disruption project of single P-element insertions reveals lethal lines mutating 25% of vital *Drosophila* genes Spradling, A. C., D. Stern, *et al.*, *Genetics* 153: 135-177 (1999).

Males carrying the transposase source P( $\Delta 2-3$ ) are crossed en masse to yellow white females homozygous for a P-lacW insertion on the X chromosome. Males carrying the PlacW insertion on the X and  $\Delta 2-3$  on the third chromosome are collected from this cross. The F0 "jumpstart" males are crossed in groups of 10-15 to 20-25 females of w spl; Sb/TM3, Ser genotype. Male F1 progeny with pigmented eyes indicate that the P-lacW has jumped to an autosome. An average of 10-15 males from each F0 cross lacking  $\Delta 2-3$  are crossed individually to y w; DTS-4/TM3, Sb Ser females, that all third chromosomal insertions result in balanced F2 stocks. Insertions on other autosomes yield white-eyed flies in the F2 generation and are eliminated. The balanced third chromosome insertions are tested for lethality in the next



generation by placing four to six pairs of y w; P-lacW/TM3, Sb Ser flies in a vial and examining their progeny for the presence of homozygous P-lacW flies. To analyze the lethal phase, the TM3, Sb Ser balancer is replaced by the TM6C, TB Sb chromosome. In such a genetic background, homozygous mutants can be identified by their wild-type body-length. An average of 10-15 pairs of flies are placed in vials supplemented with yeast paste, and the eggs are collected from each line for 1 day. The development of 50-100 progeny is monitored, and the presence of homozygotes are recorded in all developmental stages. Lethal phase is assigned to a developmental stage in which homozygote animals last appear. Lethal lines are identified and maintained.

Table 3 P-element location

seq ID	p-element line	Inverse PCR	df cross
14	l(1)G0335	516M3h-f09	Df(2L)Dwee[wo5]
16	l(3)064301	979H5h-b01	Previously verified
18	l(3)092416	1022H5h-c03	Previously verified
20	l(1)G0384	449M3h-b09	Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
22	l(1)G0449	267M3h-d07	Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
24	l(3)s126215	1082H5h-f05	GN50(63E;64B)
26	l(1)G0435	661m3h	C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+] na[+] Ste[+]
28	l(3)079101	798H5h-e01	df 084D04-06;085B06
32	l(3)s147104	1108H5h-h06	6-7(82D;82F)by62(85D;85F)
34	l(3)047418	957H5h-a05	Previously verified
36	l(1)G0425	619M5h-b-e10	Dp(1;Y)619, y[+] B[S]/w[1] otd[9]/C(1)DX, y[1] w[1] f[1]
38	l(3)122404	1079H5h-f02	Previously verified
40	l(1)G0105	360H5hA	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
44	l(3)057809	971M5h-e06	Previously Verified
46	l(1)G0127	373M3h-f03	Previously Verified
48	l(1)G0469	629H3h-f	C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+] na[+] Ste[+]
50	l(3)S070103	788M5h-h03	091F01-02;092D03-06 BL#3012
54	l(3)S104104	1057M5h-g08	Previously Verified
56	l(3)s090609	1017H5h-a03	emc5(61C;62A)
58	l(3)093909	1026H5h-a11	Previously Verified
60	l(1)G0095	354M3h-e10	Df(1)GE202/Y; Dp(1;2)sn[+]72d/Dp(?;2)bw[D], bw[D]
62	l(1)G0031	577M3h-h06	BL3219 C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+]

			na[+] Ste[+]
64	l(1)G0354	524M3h-g04	BL1319 Tp(1;2)w-ec, ec[64d] cm[1] ct[6] sn[3]/C(1)DX, y[1] w[1] f[1]
66	l(1)G0062	333H5h-b02	Df(1)R20, y[1?]/C(1)DX, y[1] w[1] f[1]/Dp(1;Y)y[+]mal[+]
74	l(2)k00237	AQ034169	BL3219 C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+] na[+] Ste[+]
76	l(1)G0181	492H3h-f	BL936 Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
78	l(3)078514	797H5h-d12	def. 087D01-02;088E05-06
80	l(3)s112110	1069H5h-e04	ry506(88B;88D)
82	l(3)024120	930H5h-e06	Previously verified
84	l(1)G0150	442M3h-b02	Df(1)R20, y[1?]/C(1)DX, y[1] w[1] f[1]/Dp(1;Y)y[+]mal[+]
88	l(3)054211	968H5h-a09	Previously verified
90	l(1)G0399	659m3h	BL 901Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
92	l(1)G0399	659m3h	BL 901Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
94	l(3)S104002	1061H5h-d08	W4(75B;75C)by62(85D;85F)
96	l(3)S133705	1092M5h-f09	Previously verified
98	l(3)041706	949H5h-g10	Previously verified
100	l(1)G0251	392M3h-f11	Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
102	l(3)100409	1050H5h-c09	crb87-5(95F;96A)
104	l(1)G0491	643M5h-b-g11	BL3219 C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+] na[+] Ste[+]
108	l(1)G0306	603m3h	BL1879 Df(1)GE202/Y; Dp(1;2)sn[+]72d/Dp(2;2)bw[D], bw[D]
112	l(1)G0344	609H5hA	BL3219 C(1;Y)1, Df(1)g, y[1] f[1] B[1]/C(1)A, y[1]/Dp(1;f)LJ9, y[+] g[+] na[+] Ste[+]
116	l(3)s083705	1006H5h-h07	2-2(81F;82F)
118	l(1)G0044	319M3h-c02	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
120	l(1)G0012	300M5h-b-e08	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
122	l(1)G0012	300M5h-b-e08	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
124	l(1)G0431	566H3h-f	BL 901 Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
126	l(1)G0130	376H3h-f-e10	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
128	l(1)G0010	576M3h-c07	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
130	l(3)s118602	1076H5h-e11	ZP1(66A;66C)G28(66B;66C)ry506(88B;88D)red1(88B;88D)
132	l(1)G0285	508H3h-f-e03	BL3033 Df(1)R20, y[1?]/C(1)DX, y[1] w[1] f[1]/Dp(1;Y)y[+]mal[+]
134	l(3)s137212	1094H5h-g05	GN50(63E;64B)
136	P{GawB}c338	F49 (13m3h	
138	l(1)G0334	515M3h-g09	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
140	l(1)G0464	627M3h-d	BL5292 (008C-D;009B + 001A01;001B02)
142	l(3)099013	1044H5h-c04	Previously Verified
144	l(3)144912	1103H5h-h01	Previously verified

146	l(1)G0345	471M3h-d03	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
148	l(1)G0453	663M3h-d03	BL5292 y[1] nej[Q7] v[1] f[1]/Dp(1;Y)FF1, y[+]/C(1)DX, y[1] w[1] f[1]
150	l(1)G038	616H5hB	BL 929 Df(1)v-L15, y[1]/C(1)DX, y[1] w[1] f[1]; Dp(1;2)v[+]75d/+
152	l(1)G0492	666M3h-d06	Previously verified
154	l(1)G0052	325M5h-b-f01	Df(1)v-N48, f[*]/Dp(1;Y)y[+]v[+]#3/C(1)DX, y[1] f[1]
156	l(1)G0269	653M5h-b	BL3033 Df(1)R20, y[1?]/C(1)DX, y[1] w[1] f[1]/Dp(1;Y)y[+]mal[+]
158	l(1)G0241	422H3h-f-d02	Dp(1;Y)BSC1, y[+]/w[67c23] P{lacW}l(1)G0060[G0060]/C(1)RM, y[1] v[1]
162	l(1)G0141	277M5h-b-b08	Dp(1;Y)BSC1, y[+]/w[67c23] P{lacW}l(1)G0060[G0060]/C(1)RM, y[1] v[1]
164	l(1)G0250	468H5h-e02	BL5292 y[1] nej[Q7] v[1] f[1]/Dp(1;Y)FF1, y[+]/C(1)DX, y[1] w[1] f[1]
166	l(3)s030003	943H5h-e09	M-Kx1(86C;87B)T-61(86E;87A)T32(86E;87C)
168	l(1)G0428	456M3h-c04	BL1538 Df(1)os[UE69]/C(1)DX, y[1] f[1]/Dp(1;Y)W39, y[+] != fcl[+]Y
170	l(3)072603	996H5h-h02	Previously verified
172	l(3)S094310	1029H5h-c08	Previously verified
174	l(1)G0220	467M3h-d02	M19 BL1527 Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
176	l(3)090417	811H5h-e11	def. 087D01-02;088E05-06
178	l(3)s2172	AQ034107	gasfilling screen
180	l(1)G0025	310M3h-d09	Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
182	l(1)G0076	343M3h-d11	Previously verified
184	l(1)G0151	482M3h-g04	BL1527 Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
186	l(3)S069605	990M5h-f06	Previously verified
188	l(1)G0221	434H3h-f-f02	Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]
190	l(1)G0075	342M3h-d12	Df(1)v-N48, f[*]/Dp(1;Y)y[+]v[+]#3/C(1)DX, y[1] f[1]
192	l(3)s002001	886H5h-c09	R-G5(62A;62D)R-G7(62B;62F)
196	l(1)G0046	321M3h-c04	Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
198	l(1)G0020	303M5h-b-f06	Dp(1;Y)619, y[+] B[S]/w[1] otd[9]/C(1)DX, y[1] w[1] f[1]
200	l(3)s095214	1032H5h-b05	faf-BP(100D;100F)
202	l(1)G0481	275H5hB	Dp(1;Y)619, y[+] B[S]/w[1] otd[9]/C(1)DX, y[1] w[1] f[1]
206	l(3)s119608	1077H5h-e12	B81(99C;100F)
208	l(1)G0172	650H3h-f-c12	BL5292 y[1] nej[Q7] v[1] f[1]/Dp(1;Y)FF1, y[+]/C(1)DX, y[1] w[1] f[1]
210	l(1)G0429	564M3h-b11	BL5459 C(1;Y)6, y[1] w[*] P{white-un4}BE1305 mew[023]/C(1)RM, y[1] pn[1] v[1]; Dp(1;Y)y[+]
212	l(3)005028	892H5h-a04	Previously verified
216	l(1)G0343	520M5h-b	BL5594 Df(1)dhd81, w[1118]/C(1)DX, y[1] f[1]; Dp(1;2)4FRDup/+
218	l(1)G0343	520M5h-b	BL5594 Df(1)dhd81, w[1118]/C(1)DX, y[1] f[1]; Dp(1;2)4FRDup/+
220	l(1)G0174	463M3h-c10	Df(1)dhd81, w[1118]/C(1)DX, y[1] f[1]; Dp(1;2)4FRDup/+
224	l(1)G0132	377H3h-f-f10	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
226	l(1)G0144	387M3h-f06	Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
228	l(1)G0144	387M3h-f06	Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
230	l(1)G0312	291M5h-b-	Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]

		g08	
232	l(3)S044402	954M5h-b06	Previously Verified
234	l(1)G0375	534M5h-b-h03	BL936 Df(1)64c18, g[1] sd[1]/Dp(1;2;Y)w[+]/C(1)DX, y[1] w[1] f[1]
236	l(1)G0159	486M3h-d09	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
238	l(1)G0227	651H3h-f	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
240	l(1)G0212	433M3h-a06	Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]
242	l(1)G0296	383H5hA	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
244	l(3)j2B9	AQ026304	gasfilling screen
248	l(1)G0007	298M3h-a08	Previously verified
250	l(3)070006	991H5h-b08	Previously verified
252	l(1)G0423	454M3h-c02	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
254	l(1)G0361	527H3h-f	BL5596 Dp(1;Y)BSC1, y[+]/w[67c23] P{lacW}l(1)G0060[G0060]/C(1)RM, y[1] v[1]
256	l(1)G0290	285H5hA	Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
258	l(1)G0436	570M3h-c03	BL 929 Df(1)v-L15, y[1]/C(1)DX, y[1] w[1] f[1]; Dp(1;2)v[+]75d/+
260	l(1)G0111	362M5hA	Dp(1;Y)BSC1, y[+]/w[67c23] P{lacW}l(1)G0060[G0060]/C(1)RM, y[1] v[1]
262	l(1)G0183	264H3h-f-e07	Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
264	l(3)S100209	1049H5h-d08	Previously verified
266	l(3)S100209	1049H5h-d08	Previously verified
268	l(1)G0438	572M3h-c05	BL5270 Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]
270	l(1)G0116	366M5h-b-f09	Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]
272	l(3)S025007	934M5h-g05	Previously verified
274	l(1)G0419	561M3h-b09	BL 929 Df(1)v-L15, y[1]/C(1)DX, y[1] w[1] f[1]; Dp(1;2)v[+]75d/+
276	l(3)S008418	900H5h-a05	Previously verified
278	l(3)141110	1098H5h-g08	Previously verified
280	l(3)S148011	1110H5h-h08	P115(89B;89E)C4(89E;90A)
282	l(3)S023204	923M5h-f05	Previously verified
284	l(3)S096404	1037H5h-a08	Previously verified
286	l(3)145511	1104H5h-h02	Previously verified
292	l(3)S110013	1066H5h-h08	Previously verified
294	l(3)010605	904H5h-d11	Previously verified
296	l(3)100604	1051H5h-c10	Previously verified
302	l(3)001604	883H5h-c06	Previously verified
304	l(1)G0358	526M3h-g06	BL1538 Df(1)os[UE69]/C(1)DX, y[1] f[1]/Dp(1;Y)W39, y[+] != fcl[+]Y
306	l(3)067006	984H5h-g07	Previously Verified
308	l(1)G0070	338M3h-d08	Df(1)os[UE69]/C(1)DX, y[1] f[1]/Dp(1;Y)W39, y[+] != fcl[+]Y
310	l(3)02240	G00700	Df(3L)AC1
312	l(3)088205	1013H5h-c01	Previously Verified

314	l(3)S042228	951H5h-f01	vin2(67F;68D)vin5(68A;69A)
316	l(3)S050407	964H5h-a07	M-Kx1(86C;87B)T-61(86E;87A)T32(86E;87C)
318	l(3)011046	908H5h-d09	Previously verified
320	l(3)S094204	1028H5h-b01	ea(88E;89A)
322	l(3)001917	738H5h-a03	def. 089E01-F04;091B01-B02
324	l(3)131602	858H5h-h10	def. 089E01-F04;091B01-B02
326	l(1)G0451	624M3h-a10	BL 901 Df(1)svr, N[spl-1] ras[2] fw[1]/Dp(1;Y)y[2]67g19.1/C(1)DX, y[1] f[1]
328	l(3)S022231	920H5h-g04	Previously verified
330	l(3)S085401	225M3d	Df(3L-Xs-533/TM6B Sb[1]Ser[1] (76B4-77B)
332	l(3)075515	794H5h-d09	def. 076B04;077B
334	l(3)131602	858H5h-h10	def. 089E01-F04;091B01-B02
336	l(3)058302	972H5h-a11	Previously verified
338	l(3)058302	972H5h-a11	Previously verified
340	l(3)S005916	895H5h-d01	lxd6(67F;68D)P14(90C;91A)
342	l(3)025616	752H5h-b02	def. 087D01-02;088E05-06
348	l(3)S089302	1014H5h-a01	AC1(67A;67D)
354	l(2)06444	AQ025653	In(2R)vg[W]
356	l(3)026115	938H5h-e07	Previously verified
358	l(1)G0461	626M3h-a12	BL5279 Df(1)JC70/Dp(1;Y)dx[+]5, y[+]/C(1)M5
360	l(2)04329	G00564	Df(2R)vg135 Df(2R)CX1
362	l(3)113105	1070H5h-e05	Previously verified
364	l(1)G0213	495M5h-b	BL1537 Dp(1;Y)W73, y[31d] B[1], f[+], B[S]/C(1)DX, y[1] f[1]/y[1] baz[EH171]
366	l(3)003606	888H5h-d06	Previously verified
368	l(3)S005042	893H5h-c01	eN19(93B;94)eR1(93B;93D)
372	l(3)S075101	1002H5h-h04	pXT103(85A;85C)
374	l(1)G0455	269H5h-a01	BL5678 duplication
376	l(1)G0260	432M3h-a05	Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]
378	l(3)S086909	806H5h-b04	087D01-02;088E05-06 BL1534
380	l(1)G0272	435H3h-f-g02	M26 BL5270 Df(1)19, f[1]/C(1)RM, y[1] shi[1] f[1]; Dp(1;Y)shi[+]3, y[+]

### Example 2: Sequence Determination

**Inverse PCR:** To determine the flanking sequence of the lethal lines, the "Inverse PCR and Cycle Sequencing Protocol for Recovery of Sequences Flanking PZ, PlacW, and PEP elements" of E. Jay Rehm, Berkeley *Drosophila* Genome Project on the world wide web at [fruitfly.org/methods/](http://fruitfly.org/methods/) is used with slight modifications. These modifications include the following: genomic DNA is obtained from 10 flies, rather than 30 flies, with adjustments for final concentrations; all DNA precipitations are performed using glycogen; for some reactions, all of the digest volume is used in the appropriate ligations; the number of cycles in PCR

reactions was increased to 40; Pry1 and Pry2 were used to sequence the PEP line flanking sequences.

**Genomic DNA isolation:** Flies are collected and frozen at  $-20^{\circ}\text{C}$  until ready for use. Genomic DNA is prepared by grinding flies in 200  $\mu\text{l}$  Buffer A with a disposable grinder 30X (Buffer A is composed of 100 mM Tris-Cl, pH7.5, 100 mM EDTA, 100 mM NaCl, 0.5% SDS). Add 200  $\mu\text{l}$  additional Buffer A; grind another 15X. Keep on ice until finished. Incubate at  $65^{\circ}\text{C}$  for 30 minutes. Vortex to mix. Add 800  $\mu\text{l}$  freshly made LiCl/KAc Solution (LiCl/ KAc Solution is comprised of 1 part 5 M KAc and 2.5 parts 6 M LiCl). Vortex. Incubate  $-20^{\circ}\text{C}$  for 20 minutes. Spin at maximum speed at room temperature 15+ minutes. Transfer 1 ml supernatant to a clean tube avoiding floating debris. Add 600  $\mu\text{l}$  room temperature isopropanol to supernatant. Mix well by tipping. Add 0.5  $\mu\text{l}$  glycogen. Vortex. Incubate at room temperature for 5 minutes. Spin 15 minutes at room temperature, maximum speed. Aspirate away the supernatant. Wash 2X with 500  $\mu\text{l}$  70% room temperature ethanol; vortex between washes. Spin for 10 minutes at room temperature, maximum speed. Aspirate away supernatant. Dry in a speed vacuum for 10 minutes. Resuspend in 50  $\mu\text{l}$  TE + 0.1 mg/ml RNase A {for 1 ml TE/RNase A Solution, add 990  $\mu\text{l}$  TE + 10  $\mu\text{l}$  RNase A (10mg/ml)}. Check 5  $\mu\text{l}$  on 0.8% gel.

**Digest Genomic DNA** (Sau3A I, HinP1 I, or Msp I--done separately): Set up digests in 96 well tray. Per reaction, add 10  $\mu\text{l}$  genomic DNA, 5  $\mu\text{l}$  10X Buffer, 2  $\mu\text{l}$  0.1mg/ml RNAase A stock, 30.5  $\mu\text{l}$  dH<sub>2</sub>O, 10 units of enzyme (8 units for Sau 3A I), 0.5 $\mu\text{l}$  of 100X BSA (for Sau 3AI only). Incubate at  $37^{\circ}\text{C}$  for 2.5 hours. Check on 0.8% gel before heat-inactivating at  $65^{\circ}\text{C}$  for 20 minutes.

**Ligate P Element and Flanking DNA:** Set-up ligation tube with 400  $\mu\text{l}$  of ligation mixture then add 30-50  $\mu\text{l}$  of the digest: Per reaction, add 30  $\mu\text{l}$  of digested genomic DNA, 43  $\mu\text{l}$  of 10X ligation buffer (NEB), 375  $\mu\text{l}$  of dH<sub>2</sub>O, and 2  $\mu\text{l}$  of ligase (2 Weiss units). Incubate overnight at  $4^{\circ}\text{C}$ . Total reaction volume is adjusted as appropriate.

**Precipitate Ligated DNA:** To ligation tube, add 40  $\mu\text{l}$  3M NaAc pH5.2 + 1ml 100% room temperature ethanol + 1  $\mu\text{l}$  glycogen. Mix by tipping. Incubate  $-20^{\circ}\text{C}$  for 15+ minutes. Spin 15 minutes,  $4^{\circ}\text{C}$ . Aspirate away supernatant. Wash with 500  $\mu\text{l}$  room temperature 70% ethanol. Vortex. Spin room at temperature for 10 minutes. Aspirate away supernatant. Dry in speed vacuum for 10 minutes. Resuspend in 50  $\mu\text{l}$  TE. Vortex to mix. Transfer to 96 well plate.

PCR: Set up PCR reactions in 96 well plates (Applied Biosystems). Set up PCR reactions with primers appropriate for the type of P element and the end of the element from which genomic sequence is to be recovered.

Primers for PCR: (type of P element 5' or 3' end forward primer reverse primer annealing temperature):

PZ P-element5' endPlac4Plac1	60°
PZ P-element3' endPry4Pry1	55°
PZ P-element3' endPry2Pry1	60°
PlacW P-element5' endPlac4Plac1	60°
PlacW P-element3' endPry4Plw3-1	55°
PlacW P-element3' endPry2Pry1	60°
PEP P-element5' endPwht1Plac1	60°
PEP P-element3' endPry4Pry1	55°
PEP P-element3' endPry2Pry1	60°

The Pry2/Pry1 combination has a higher annealing temperature than the Pry4/Pry1 and Pry4/Plw3-1 combinations, but the resulting PCR products do not allow sequencing directly off the 3' end of the P-element. The latter primer combinations are therefore used in all initial experiments; the Pry2/Pry1 combination can be used in those cases where strong and unique bands do not result.

Per reaction: 10 µl of ligated genomic DNA, 1 µl of 10mM dNTP mix, 1 µl of 10 µM forward primer stock, 1 µl of 10 µM reverse primer stock, 5 µl of 10X Qiagen Taq buffer, 31.5 µl of dH<sub>2</sub>O, 0.5 µl of Qiagen Taq.

Cycles: 1X 95°C for 5 minutes; 40X (95°C for 30 seconds; 60°C (high temp) or 55°C (low temp) for 30 seconds; 68°C for 2 minutes); 1X 72°C for 10 minutes; hold at 4°C; run 10µl on 1.5% gel to check. Rearray positive wells to 96 well plate for sequencing clean-up. The primer sets for PCR are as shown in the table below:

Table 4 PCR Primers

Digest, End, Temperature	Forward PCR Primer	Reverse PCR Primer
H5h	Plac4	Plac1
H3h	Pry2	Pry1
H3l	Pry4	Plw3-1
M5h	Plac4	Plac1
M3h	Pry2	Pry1
M3l	Pry4	Plw3-1
S5h	Plac4	Plac1
S3h	Pry2	Pry1
S3l	Pry4	Plw3-1

## PCR Primer Sequences (5' to 3'):

Plac4 (27)	- act gtg cgt tag gtc ctg ttc att gtt	SEQ ID NO:1
Plac1 (24)	- cac cca agg ctc tgc tcc cac aat	SEQ ID NO:2
Pry4 (23)	- caa tca tat cgc tgt ctc act ca	SEQ ID NO:3
Pry1 (26)	- cct tag cat gtc cgt ggg gtt tga at	SEQ ID NO:4
Pry2 (28)	- ctt gcc gac ggg acc acc tta tgt tat t	SEQ ID NO:5
Plw3-1 (19)	- tgt cgg cgt cat caa ctc c	SEQ ID NO:6
Pwht1 (19)	- gta acg cta atc act ccg aac agg tca ca	SEQ ID NO:7

**Enzymatic Clean-Up for Sequencing:** To 40  $\mu$ l PCR reaction, add 4  $\mu$ l of enzyme mix. Incubate at 37°C for 1 hour. Inactivate at 70°C for 10 minutes. (Enzyme Mix consists of 2.5U/ $\mu$ l Exonuclease I (Amersham E700732), 0.5U/ $\mu$ l Shrimp Alkaline Phosphatase (Amersham E70183), 1X Amplitaq PCR buffer, add dH<sub>2</sub>O to final volume.)

## Example 3: Sequence Analysis

Sequence of the flanking sequence generated by inverse PCR is performed on an ABI 3700 sequencer (Perkin Elmer) using BIG DYE sequencing reaction.

Primer sets for sequencing are as shown in the table below:



Table 5 PCR Primers for Flanking Sequences

Digest, End, Temperature	Forward Primer	Reverse Primer
H5h	Splac2	Sp1
H3h	Pry2	Sp5
H3l	Spep1	Sp5
M5h	Splac2	Sp1
M3h	Pry2	Sp5
M3l	Spep1	Sp5
S5h	Splac2	Sp1
S3h	Pry2	Sp6
S3l	Spep1	Sp6

The following primer sets are designed to sequence both ends of PCR products recovered from PlacW and PZ strains:

Splac2 and Sp1 - for use with the Plac4/Plac1 5' PCR primer combination with either PZ or PlacW P-elements; allows sequencing of both ends of the PCR fragment.

Spep1 and Sp3 - for use with the Pry4/Pry1 3' PCR primer combination with PZ P-elements; allows sequencing of both ends of the PCR fragment.

Spep1 and Sp6 - for use with the Pry4/Plw3-1 3' PCR primer combination with PlacW P-elements where Sau3a digestion is performed; allows sequencing of both ends of the PCR fragment.

Spep1 and Sp5 - for use with the Pry4/Plw3-1 3' PCR primer combination where HinP1 digestion is performed; allows sequencing of both ends of the PCR fragment.

Pry1 and Pry2 - for use with the Pry1/Pry2 3' PCR primer combination; allows sequencing of both ends of the PCR fragment.

The PCR products recovered from PEP strains are sequenced with the following primers: Sp1- for use with the Pwht1/Plac1 5' PCR primer combination with the PEP element; Spep1- for use with the Pry4/Pry1 3' PCR primer combination with the PEP element; Pry1 and Pry2 for use with the Pry1/Pry2 3' PCR primer combination with the PEP element.

Primer Sequences (5' to 3'):

Splac2 (25)	- gaa ttc act ggc cgt cgt ttt aca a	SEQ ID NO:8
Sp1 (22)	- aca caa cct ttc ctc tca aca a	SEQ ID NO:9
Sp3 (24)	- gag tac gca aag ctt taa cta tgt	SEQ ID NO:10
Sp6 (23)	- tga cca cat cca aac atc ctc tt	SEQ ID NO:11
Sp5 (25)	- gca tca caa aaa tcg acg ctc aag t	SEQ ID NO:12
Spep1 (19)	- gac act cag aat act att c	SEQ ID NO:13

Melting temperatures of sequencing primers:

Splac2- 60.1°C

Sp1- 50.6°C

Sp3- 49.3°C

Sp6- 54.9°C

Sp5 -60.3°C

Spep1- 44.8°C

#### Example 4: Secondary Confirmation of Lethality

The lethality of the chromosome carrying the P-element insertion is demonstrated genetically as described in Example 1. The essential *Drosophila* nucleotide sequences are identified by isolating nucleotide sequences flanking the P-element insertion and aligning those sequences with genomic *Drosophila* sequence obtained from the Celera *Drosophila* database. However, in some instances, a second site mutation exists on the chromosome that is responsible for the lethality. In other instances, the location of the flanking sequence is such that determination of which gene(s) are affected by the P-element insertion is rendered difficult or impossible. Thus, to provide secondary confirmation that the gene indicated is essential, there are many methods that one skilled in the art can use, e.g., rescue of the lethality using transformation technology, perturbation of the gene in a targeted manner, or failure to complement a deficiency.

To provide secondary confirmation, lethal lines are crossed to a line containing a deficiency. This creates a hemizygous condition in that particular region and reveals the recessive phenotype of the P-element. Complementation with deficiencies that unequivocally remove the P-element insertion site is taken as proof that the P-element does not cause the

associated phenotype. Failure to complement indicates that the strain is verified. This method is as performed in Spradling, A. C., D. Stern, *et al.*, *Genetics* 153: 135-177 (1999). If the insert is present on the X chromosome, which is present in two copies in females but only one copy in males, then the recessive phenotype of the P-element insert is revealed by this hemizygous condition in males. A rescue cross is performed to a stock containing a duplication spanning the region of the insert on the X chromosome on one of the autosomes. If the males survive then the presence of an essential gene disrupted by the P-element but rescued by the duplication is confirmed. While lines with secondary mutations closely linked to the P insertion might be erroneously verified by these procedures, further molecular and genetic analyses suggest that the frequency of such errors is small. RNA interference, described in Fire, A., S. Xu, *et al.*, *Nature* 391, 806-811 (1998) and Kennerdell, J.R. and Carthew, R.W., *Cell* 95, 1017-1026 (1998), is used as a method to target a gene of interest and demonstrate that the perturbation of the identified gene produces a lethal phenotype.

#### Example 4a: Double-Stranded RNA Interference

Preparation of dsRNA for Injection. Sequences to be expressed as dsRNA were cloned into Bluescript KS(+) (Stratagene of La Jolla, California), linearized with the appropriate restriction enzymes, and transcribed *in vitro* with the Ambion T3 and T7 Megascript kits following the manufacturer's instructions (Ambion Inc. of Austin, Texas). Transcripts were annealed in injection buffer (0.1mM NaPO<sub>4</sub> pH 7.8, 5mM KCl) after heating to 85°C and cooling to room temperature over a 1- to 24-hr period. All annealed transcripts were analyzed on agarose gels with DNA markers to confirm the size of the annealed RNA and quantitated as described previously (Fire et al. (1998) *Nature* 391(6669):806-811). Injected RNA was not gel-purified. Injection of 0.1 nl of a 0.1- to 1.0-mg/ml solution of a 1-kb dsRNA corresponds to roughly 10<sup>7</sup> molecules/injection.

Injection of *Drosophila melanogaster* Embryos. Fly cages were set up using 2- to 4-day flies. Agar-grape juice plates were replaced every hour to synchronize the egg collection for 1-2 days. The eggs were collected over a 30- to 60-min period for subsequent injection. The eggs were washed into a nylon mesh basket with tap water. The chorion was removed by brief

soaking in a dilute bleach solution. Eggs were positioned on a glass slide such that each egg was in a same orientation. Double-stranded RNA was injected into middle of each egg using an Eppendorf transjector (Eppendorf Scientific, Inc. of Westbury, New York). Following injection, slides were stored in a moist chamber to prevent dessication of the embryos. Embryos were monitored for development and transferred as first instar larvae to vials containing *Drosophila* medium. Methods for rearing *Drosophila* staging and common genetic techniques can be found, for example, in Roberts (1986) *Drosophila melanogaster, A Practical Approach*, IRL Press, Washington, DC; Ashburner (1989a) *Drosophila: A Laboratory Handbook*, Cold Spring Harbor Laboratory Press, New York, New York; Ashburner (1989b) *Drosophila: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, New York; Goldstein & Fyrberg, eds (1994) in *Methods in Cell Biology*, Vol. 44, Academic Press, San Diego, California.

The data in Table 6 demonstrates the lethal effect of disrupting the production of protein from the message of the specified gene through RNAi. Based on data from positive and negative controls, a reduction in survival (%viable adults from developed eggs) below 38% represents a significant lethal effect. Many genes show a complete loss of survivability (with 0% viable). Others show a range of phenotypic penetrance, which is most likely due to the variability of the RNAi technique, but are still considered lethals because they are significantly below controls.

Table 6 Data for dsRNA Interference

seq ID	Inventor's reference	# eggs injected	# eggs showing morphological development	# hatched larvae	# pupae	# adults	% viable adults from developed eggs
	none, buffer only	941	806	580	500	433	53.72
14	GIN00231,CT28483	163	148	107	28	26	17.57
30	GIN00961,CT31117	472	386	170	8	1	0.26
42	GIN01243,CT36241	107	99	81	9	7	7.07
52	GIN01682,CT1465	140	127	87	23	15	11.81
68	GIN01885,CT13424	170	154	73	17	8	5.19
70	GIN01896,CT14932	164	140	78	44	38	27.14
72	GIN01977,CT23511	79	70	18	17	15	21.43
86	GIN02340,CT28931	190	159	0	0	0	0.00
106	GIN03775,CT33819	172	148	16	0	0	0.00
110	GIN03797,CT33841	136	127	12	0	0	0.00
114	GIN04053,CT3509	168	145	106	1	1	0.69
160	GIN05757,CT4810	159	144	109	37	32	22.22
194	GIN07111,CT6007	159	140	94	0	0	0.00

204	GIN07278,CT6738	174	166	7	3	1	0.60
214	GIN07446,CT9021	125	119	1	0	0	0.00
222	GIN07609,CT6171	372	316	119	0	0	0.00
246	GIN08205,CT12517	717	569	433	26	25	4.39
274	GIN08858,CT14874	177	161	13	3	3	1.86
288	GIN09788,CT17938	100	83	71	5	2	2.41
290	GIN09819,CT17971	181	142	107	7	1	0.70
298	GIN10338,CT19788	170	137	88	5	1	0.73
300	GIN10364,CT19850	58	55	47	14	6	10.91
344	GIN11831,CT24122	103	87	0	0	0	0.00
346	GIN11918,CT24346	469	408	301	257	88	21.57
350	GIN11993,CT24437	145	130	93	0	0	0.00
352	GIN12074,CT18257	104	93	80	3	3	3.23
354	GIN12174,CT24731	168	145	122	1	1	0.69
360	GIN12437,CT25274	473	424	334	237	63	14.86
370	GIN13270,CT27543	101	92	78	2	2	2.17

#### Example 5: Isolation Of Full Length cDNA

A cDNA screen is performed using a *Drosophila melanogaster* cDNA library probed with a portion of each nucleotide sequence disclosed in the Sequence Listing. Positive colonies are selected, a subset sequenced, and a clone corresponding to the full-length cDNA is recovered. Alternatively, primers from the predicted 5' and 3' end are used in polymerase chain reaction with either a *Drosophila* cDNA library or first strand cDNAs obtained by reverse transcription of *Drosophila* mRNAs as template to amplify a fragment representing the full-length clone.

#### Example 6: Expression Of Recombinant Protein In Insect Cells

Baculovirus vectors, which are derived from the genome of AcNPV virus, are designed to provide high levels of expression of cDNA in the SF9 line of insect cells (ATCC CRL# 1711). Recombinant baculovirus expressing the cDNA of the present invention is produced by the following standard methods (InVitrogen MaxBac Manual): cDNA constructs are ligated into the polyhedrin gene in a variety of baculovirus transfer vectors, including the pAC360 and the BleBac vector (InVitrogen). Recombinant baculoviruses are generated by homologous recombination following co-transfection of the baculovirus transfer vector and linearized AcNPV genomic DNA (Kitts, P.A., *Nucleic Acid. Res.* 18: 5667 (1990)) into SF9 cells. Recombinant pAC360 viruses are identified by the absence of inclusion bodies in infected cells and recombinant pBlueBac viruses are identified on the basis of B-galactosidase expression

(Summers, M.D. and Smith, G.E., Texas Agriculture Exp. Station Bulletin No. 1555). Following plaque purification, the *Drosophila* cDNA expression is measured.

The cDNA encoding the entire open reading frame for the *Drosophila* cDNA is inserted into the BamHI site of pBlueBacII. Constructs in the positive orientation, which are identified by sequence analysis, are used to transfect SF9 cells in the presence of linear AcNPV wild type DNA. Authentic, active *Drosophila* cDNA is found in the cytoplasm of infected cells. Active *Drosophila* cDNA is extracted from infected cells by hypotonic or detergent lysis.

#### Example 7: Expression Of Recombinant Protein In *E. coli*

A cDNA clone of the present invention is subcloned into an appropriate expression vector and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the recombinant protein is confirmed. Recombinant protein is then isolated using standard techniques.

#### Example 8: *In vitro* Binding Assays

Recombinant protein is obtained, for example according to Example 6 or Example 7. The protein is immobilized on chips appropriate for ligand binding assays. The protein immobilized on the chip is exposed to sample compound in solution according to methods well known in the art. While the sample compound is in contact with the immobilized protein measurements capable of detecting protein-ligand interactions are conducted. Examples of such measurements are SEDLI, biacore and FCS, described above. Compounds found to bind the protein are readily discovered in this fashion and are subjected to further characterization.

The above disclosed embodiments are illustrative. This disclosure of the invention will place one skilled in the art in possession of many variations of the invention. All such obvious and foreseeable variations are intended to be encompassed by the appended claims.

The numerous publications and patents referred to in this document are hereby incorporated by reference, in their entirety.